Australian Government
Department of Agriculture, Fisheries and Forestry

Review of the biosecurity risks of prawns imported from all countries for human consumption

Final report

Animal Biosecurity Branch

Cataloguing data

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Acknowledgement of Country

We acknowledge the Traditional Custodians of Australia and their continuing connection to land and sea, waters, environment and community. We pay our respects to the Traditional Custodians of the lands we live and work on, their culture, and their Elders past and present.

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Summary

The Australian Government Department of Agriculture, Fisheries and Forestry (the department) has conducted this risk review to assess the biosecurity risks associated with the import of prawns from all countries for human consumption.

This risk review considers scientific information, advice from international scientific experts, relevant industry practices and operational practicalities.

Australia currently permits the importation of prawns providing they meet specific import conditions. This risk review proposes that prawns continue to be permitted import into Australia, subject to a range of biosecurity measures.

This risk review identified hazards that require biosecurity measures to manage the risks to a very low level to achieve Australia's appropriate level of protection (ALOP).

Table 1 provides a summary of whether the biosecurity measures (from least restrictive to most restrictive) manage biosecurity risk for each hazard (refer Table 26 for the underpinning risk assessment values for each hazard and biosecurity measure).

Table 1 Summary of the biosecurity measures and how they achieve Australia's appropriate level of protection for each hazard for imported frozen prawns

Hazard	Biosecurity measures and whether their use on their own achieves Australia's appropriate level of protection (yes/no/na)								
	Labelling	Unrestricted (frozen)	Head & shell removal	Head & shell removal + deveining	Head & shell removal + testing	Head & shell removal + 2x testing	Cooking	Value-added product	Free populations
WSSV	no	no	no	no	no	yes	yes	yes	yes
YHV1	no	no	no	no	no	yes	yes	yes	yes
EHP	no	no	no	yes	na	na	yes	yes	yes
CMNV	no	no	yes	na	na	na	yes	yes	yes
DIV1	no	no	yes	na	na	na	yes	yes	yes
TSV	no	no	yes	na	na	na	yes	yes	yes
IMNV	no	no	yes	na	na	na	yes	yes	yes
LSNV	no	no	yes	na	na	na	yes	yes	yes
Vp AHPND	no	no	yes	na	na	na	yes	yes	yes
"*Ca*. H. penaei"	no	yes	na	na	na	na	yes	yes	yes

na not assessed because less restrictive biosecurity measures achieve Australia's appropriate level of protection for imported frozen prawns. **Hazard: WSSV** white spot syndrome virus. **YHV1** yellow head virus genotype 1. **EHP** *Enterocytozoon hepatopenaei*. **CMNV** covert mortality nodavirus. **DIV1** decapod iridescent virus 1. **TSV** Taura syndrome

virus. **IMNV** infectious myonecrosis virus. **LSNV** Laem-Singh virus. ***Vp* AHPND** *Vibrio parahaemolyticus* strains containing Pir toxins. **"Ca. H. penaei "** "*Candidatus* Hepatobacter penaei". **Biosecurity measures: Labelling** packages marked with the words "*For human consumption only. Not to be used as bait or feed for aquatic animals*". **Unrestricted** whole, uncooked frozen prawns with no biosecurity measures applied. **Head & shell removal** uncooked frozen prawns which have had the head and shell removed ((last tail segment and tail fans permitted). **Head & shell removal + deveining** uncooked frozen prawns which have had the head and shell removed ((last tail segment and tail fans permitted) and been deveined (removal of the digestive tract to at least the last tail segment). **Head & shell removal + testing** uncooked frozen prawns which have had the head and shell removed, been deveined and batch tested pre-export. **Head & shell removal + 2x testing** uncooked frozen prawns which have had the head and shell removed, been deveined and batch tested pre-export and on-arrival. **Cooking** frozen prawns which have been cooked so that they appear fully cooked and have achieved a core temperature of at least 65°C. **Value-added products** encompasses breaded, battered and crumbed prawns, and dumpling and dim sum type-products containing uncooked frozen prawns which have had the head and shell removed ((last tail segment and tail fans permitted). **Free populations** frozen prawns which have been sourced from a country, compartment or zone that is recognised by Australia to be free of the pathogenic agent.

The department recognises that there might be new scientific information and technologies, or other combinations of measures that may provide an equivalent level of biosecurity protection for the hazards identified as requiring risk management in this report. Submissions supporting equivalence measures will be considered on a case-by-case basis.

This final report contains details of the risk review for each hazard and the proposed biosecurity measures to manage identified risks.

1 Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity system consists of three focus areas for preventing or responding to the incursion of pests and diseases: overseas; at our border and within Australia. Across these three focus areas, the Australian Government Department of Agriculture, Fisheries and Forestry (the department) undertakes a range of policy, operational and compliance functions and implements a various education, awareness and communication campaigns.

Biosecurity risk cannot be reduced to zero at our border. The success of the national biosecurity system in protecting Australia's environment, economy and way of life relies on the efforts of all parties and is a shared responsibility. The department works across the Commonwealth and with governments, industry, research institutions and community groups to implement improvements across the system to manage biosecurity risk efficiently and effectively.

The risk analysis process is an important part of Australia's biosecurity system. It enables the Australian Government to formally consider the level of biosecurity risk that may be associated with proposals to import goods into Australia. If the biosecurity risks do not achieve Australia's appropriate level of protection (ALOP), biosecurity measures are proposed to reduce the risks to an acceptable level. If the risks cannot be reduced to an acceptable level, the goods will not be imported into Australia until suitable measures are identified.

Successive Australian Governments have maintained a conservative, but not a zero risk, approach to the management of biosecurity risks. This approach is expressed in terms of Australia's ALOP, which is described as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

1.1.1 Australia's appropriate level of protection

As per our international obligations, Australia applies ALOP in a consistent way across all products (that is, aquatic animal, terrestrial animal, and plant products). The biosecurity risks associated with imported products are assessed through a science-based process. As unique risk factors and scenarios apply to each product, those risks are managed in different ways to ensure that Australia's ALOP is achieved. They also consider the World Organisation for Animal Health (WOAH, formerly OIE) recommendations for managing biosecurity risk associated with the product. Importantly, biosecurity measures are selected based on whether they reduce risk to a level that achieves Australia's ALOP. Australia imposes conditions above those recommended by the WOAH for uncooked prawns for human consumption. We do this in line with our international rights and obligations and supported by risk assessments.

1.2 This risk review

The department undertakes risk analyses using technical and scientific experts in relevant fields and involves consultation with stakeholders at various stages during the process.

Risk analyses may be conducted as a biosecurity import risk analysis (BIRA) or a non-regulated risk analysis. A BIRA is a risk analysis with key steps that are conducted by the department under the Biosecurity Act 2015. A BIRA only occurs in cases where relevant risk management measures have

either not been established or exist for a similar good and pest or disease combination, but the likelihood and/or consequences of entry, establishment or spread of pests or diseases could differ significantly from those previously assessed.

A risk analysis which does not meet the criteria of a BIRA is undertaken as a non-regulated analysis of existing policy. Non-regulated risk analyses include scientific reviews of existing policy or import conditions (such as this risk review) and reviews of biosecurity measures in light of new scientific information. Non-regulated risk analyses are undertaken through an administrative process which is not provided for in law, however they still meet Australia's international rights and obligations. Non-regulated risk analyses use a similar technical methodology as BIRAs. It is important to note that not all non-regulated risk analyses are undertaken as a formal risk review such as this one. Notifications are only given to stakeholders through Biosecurity Advice notices for significant or complex scientific reviews of existing policy. Less significant or complex risk reviews may not be released publicly unless the review determines that changes need to be made to import conditions. Further information about Australia's biosecurity framework is provided in the *Biosecurity import risk analysis guidelines 2016* located on the department's website.

1.2.1 Background

The department released Biosecurity Advice 2017-07 announcing the *Review of the biosecurity risks of prawns imported from all countries for human consumption*, on 16 May 2017 (Department of Agriculture and Water Resources 2017a). This risk review is conducted as a non-regulated risk analysis of the existing import conditions and policy, including the *Generic import risk analysis report for prawns and prawn products 2009* (Prawn IRA 2009) (Biosecurity Australia 2009).

This risk review commenced in response to the white spot disease (WSD) outbreak that occurred in South-East Queensland in 2016–17 and in recognition of emerging diseases and advances in scientific knowledge since the release of the Prawn IRA 2009. Following the WSD outbreak, the department determined that the biosecurity risks of uncooked prawns imported to Australia for human consumption, under the import conditions in place at that time, was above Australia's ALOP and a 6 month suspension was placed on the import of uncooked prawns on 6 January 2017.

1.2.2 Scope

The scope of this risk review is to consider the biosecurity risk associated with the import of prawns from all countries for human consumption. The 'unrestricted commodity' (or single-entry scenario) in the Prawn IRA 2009 was '*non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption*' as that commodity represented the highest biosecurity risk. This commodity is still considered to represent the highest biosecurity risk. This risk review therefore takes the same approach as the Prawn IRA 2009 by considering the 'unrestricted commodity' to be '*non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption*'. The term 'imported prawns' is used throughout this document rather than the full description of the single-entry scenario. The type of prawn product is stated where it is relevant.

Currently Australia does not receive any chilled uncooked prawns. There are two main reasons for this. Firstly, export of chilled uncooked product to Australia is generally not practical (due to food safety and logistical reasons). Secondly, a country must be free of infection with "*Candidatus* Hepatobacter penaei" (previously known as necrotising hepatopancreatitis bacterium, causative agent of necrotising hepatopancreatitis) to export chilled uncooked prawns to Australia. To

date, no country has requested Australia recognise their freedom from "*Ca.* H. penaei" and therefore chilled uncooked prawns are not permitted import to Australia. Therefore, this risk review considers the single-entry scenario to be frozen, uncooked, whole prawns.

It is noted that there are shelf-stable food products (for human consumption) which contain prawns that this risk review does not cover. Shelf-stable food products containing prawns such as dried prawns, canned prawns or condiments containing prawns as an ingredient (for example, shelf-stable prawn paste or prawn balachan) are considered to pose a negligible risk because live crustaceans in Australia are highly unlikely to be exposed to them due to level of processing the products have undergone. Such products are not subject to the biosecurity measures recommended in this report.

A country must confirm they can meet Australia's import requirements and provide a copy of an official health certificate before they are considered an 'approved country' for the export of prawns to Australia. Aside from negotiations associated with health certificates, there is no formal assessment of the country for export of prawns to Australia under generic import conditions. Following the resumption of trade in uncooked prawns in July 2017, the department undertook expert familiarisation visits to most countries eligible to export prawns to Australia. The visits allowed the department to gather information about the aquatic animal health controls and systems in place to meet Australia's enhanced import conditions for prawns in the exporting country. This risk review is generic in nature and considers prawns imported from all countries, not just the current 'approved countries'. It also assumes that the hazards are present in all countries. Australia's import conditions for uncooked prawns assume that all supply countries have all hazards unless demonstrated to be free through departmental assessment. This assumption accounts for possible movements of prawns between countries prior to their export to Australia. Recognition of individual country disease status and sourcing from wild fisheries for export are biosecurity measures considered separately and require a formal assessment. Countries may seek Australia's assessment and recognition of disease freedom at country, zone or compartment level. Under these arrangements the department will assess a competent authority's systems to assure traceability back to source (wild or farmed populations) along with applying verification testing to provide additional disease assurances through the CBIS (compliance-based intervention scheme).

Prawns (also known as shrimp) are decapods of suborder Dendrobranchiata (Decapoda) and infraorder Caridea (Pleocyemata: Decapoda). The department does not recognise glass sponge shrimp and coral shrimp (Stenopodidea: Pleocyemata: Decapoda) as prawns relevant to the scope of this risk review as neither of these are consumed for food.

1.2.3 Existing policy

Import policy

Import policy exists for prawns from those countries approved by the department to export prawns to Australia. Table 2 shows the progressive changes made by the department to the import requirements for imported prawns since the suspension on uncooked prawn imports ended in July 2017. These import conditions will remain in place until the risk review is finalised. The biosecurity measures recommended in the final report will be the basis for the import conditions and any import permits issued.

Table 2 Changes to import conditions for prawns implemented since the white spot disease outbreak in 2016–17

Date	Changes to import conditions	Justification	Link to conditions
7 July 2017	Changed import conditions for uncooked prawns, including: • pre-export and on-arrival testing for white spot syndrome virus (WSSV) and yellow head virus genotype 1 (YHV1) • marinated prawns moved into uncooked prawn category.	Following the white spot disease outbreak in 2016–17, the department determined that the biosecurity risks of uncooked prawns under the import conditions in place at that time, did not achieve Australia's ALOP. On 6 January 2017, the Director of Biosecurity signed the *Biosecurity* (Suspended Goods – Uncooked Prawns) *Determination 2017* which suspended imports of uncooked prawns for a period of six months. The import suspension lapsed on 6 July 2017. The enhanced import conditions were implemented on 7 July 2017 to allow for safe trade. They remain in place while the department undertakes this risk review. Prior to these import conditions being implemented, uncooked prawns were only subject to on-arrival testing in Australia for WSSV and YHV1. Marinated prawns were not subject to any disease testing.	Biosecurity Advice 2017-12 (Department of Agriculture and Water Resources 2017b)
28 September 2018	Changed import conditions for breaded, battered and crumbed prawns (BBC), including: • requirement for product to be par-cooked.	New import conditions for BBC prawns were implemented in response to new information about trade trends and biosecurity risks and to ensure that imported BBC prawns continue to meet Australia's ALOP. The new conditions manage the risks associated with the increased volume of BBC products imported to Australia. The increased volume also increases the associated risks for product diversion to unintended end-uses such as fishing bait or berley, or feed for aquatic animals.	Biosecurity Advice 2018-15 (Department of Agriculture and Water Resources 2018b)
1 July 2020	New import conditions for uncooked prawns, including: • deveining of all uncooked prawns.	Whilst conducting the review, an unacceptable biosecurity risk associated with *Enterocytozoon hepatopenaei* EHP was identified. Deveining of all uncooked prawns was implemented to manage biosecurity risk for EHP to a level that achieves Australia's ALOP.	Animal Biosecurity Advice 2020-A03 (Department of Agriculture 2020)

The current import requirements for prawns are on the department's website in Animal Biosecurity Advice 2020-A03 (Department of Agriculture 2020) and on the Australian Biosecurity Import Conditions (BICON) website.

Domestic arrangements

The Australian Government is responsible for regulating the movement of animals and animal products into and out of Australia. However, the state and territory governments are responsible for animal health and environmental controls within their individual jurisdictions.

Once Australian Government biosecurity officers have cleared imported animals and animal products, they may be subject to interstate movement conditions. The importer is responsible for ensuring compliance with all requirements.

1.2.4 Consultation

To initiate stakeholder engagement in this risk review, the department hosted a roundtable discussion with key industry stakeholders on 8 February 2018. The roundtable provided stakeholders with an opportunity to discuss the risk analysis and future direction of the review, ask questions, hear differing views, and voice any concerns they may have had about the risk analysis process.

On 26 March 2018, Biosecurity Advice 2018-06 (Department of Agriculture and Water Resources 2018a) invited stakeholders to provide scientific submissions on specific issues with Australia's current prawn import conditions and the Prawn IRA 2009. Those submissions were considered when conducting the risk assessments and preparing the *Review of the biosecurity risks of prawns imported from all countries for human consumption – draft report* (draft report). Submissions which the department has been given permission to publish and the draft report are available on the prawn review webpage.

During preparation of the draft report, the department sought input from state and territory governments regarding prawn disease control and movement restrictions for prawns and prawn products within their jurisdiction. Additional information was sought on crustacean aquaculture regulation and practices, and waste disposal within their jurisdiction. The Australian Prawn Farmers Association provided information on current prawn aquaculture practices in Australia.

The Department of Health was consulted to ensure that public health considerations were included in the development of biosecurity policies. Consultation with the Australian Chief Veterinary Officer was also carried out before the release of the draft report.

In addition, the draft report was peer-reviewed by two leading independent experts in crustacean diseases, to ensure that the methods and assumptions were appropriate and that the data and information were the best available.

On 28 September 2020, the department released Animal Biosecurity Advice 2020-A05 inviting stakeholders to comment on the draft report during a 110 day consultation period. In response, the department received 17 submissions from a wide range of stakeholders.

On 24 November 2020 the department hosted a webinar to discuss the draft report. The webinar provided participants with an opportunity to hear from the department, to ask questions and to make comments about the draft report. The presentation, and a transcript of the presentation and the question-and-answer session from the webinar were published on the prawn review webpage.

1.2.5 Review of the draft and provisional final reports by the members of the scientific advisory group

On 19 February 2021, the then Minister for Agriculture, Drought and Emergency Management, the Hon David Littleproud MP announced, that the department was engaging members of the scientific advisory group (SAG) in their capacity as an independent panel of experts to review whether the department had properly considered relevant matters and made appropriate conclusions in the draft report. The expert panel provided their report to the department on 30 April 2021. The expert panel was supportive of the outcomes of the draft report and did not recommend changes to the biosecurity measures for imported prawns. The expert panel made nine recommendations, along with a range of suggestions, which are detailed in Appendix A, for the department to consider when

preparing the provisional final report. On 30 September 2022, the department provided the expert panel with the provisional final report for their review. On 3 November 2022 the expert panel provided their report to the department. The expert panel noted that the provisional final report was an extensive update of the draft report, commended the department on its revisions and noted that the:

- department had appropriately considered the findings on the expert panel report
- department had appropriately considered the stakeholder submissions received in response to the draft report
- department had included and properly considered all new scientific evidence received since the draft report was prepared
- conclusions of the provisional final report are scientifically reasonable and based on the material presented and available.

The report noted that a few minor issues remained for the department to consider when preparing the final report, with a view to improving clarity. These issues are provided in Appendix A. Both expert panel reports can be accessed on the prawn review webpage.

1.2.6 Next steps

This final report has been prepared considering the recommendations in the expert panel reviews of the draft and provisional reports, stakeholder submissions received in response to the draft report, any new information, and the outcomes of the surveys about the use of prawns for bait or berley by recreational fishers. Appendix B outlines the key issues raised by stakeholders and the way the department has addressed them in this report.

Publication of this final report represents the end of the process. The biosecurity measures recommended in the final report will be the basis for the import conditions and any import permits issued.

1.3 Australia's prawn industry

Gold Coast, Bundaberg, Mackay, Townsville and Cairns regions of Queensland (State of Queensland 2021), as well as Yamba in New South Wales are the primary locations for current prawn farming activities. In Queensland, the farms are situated singly or in small groups along the approximately 2,000 km eastern coastline.

The prawn fishing industry is the second largest fishing sector in Australia. Australia's prawn fisheries are spread around Australia and include the Northern Prawn Fishery (4,923 tonnes), Queensland East Coast fishery (4,915 tonnes), the Shark Bay and Exmouth fisheries in Western Australia (2,325 tonnes), the Gulf of St Vincent, Spencer Gulf and West Coast fisheries in South Australia (2,018 tonnes) and the New South Wales Coast fishery (1,744 tonnes) (Tuynman & Dylewski 2022).

Figure 1 depicts a map of Australia's prawn farming regions and key prawn fisheries.

Figure 1 Map of Australia's prawn farming and key prawn fisheries

Source: Modified from shutterstock.com

1.3.1 Aquaculture production

In Australia, the main prawn aquaculture species are *Penaeus monodon* and *Penaeus merguiensis* (Australian Prawn Farmers Association 2019; State of Queensland 2021).

The Australian prawn farming industry produces over 8,700 tonnes (2020–21) of product annually with a value estimated at over AU$159 million (2020–21) (Tuynman & Dylewski 2022). Farming of *P. monodon* accounts for the majority of prawn farming production in Australia. Aquaculture contributes 34.9% of total Australian prawn production volume (Tuynman & Dylewski 2022). The Australian Bureau of Agricultural and Resource Economics and Sciences have projected the value of Australian prawn production to rise in 2021–22. An increase in global prices for prawns was reported in late-2021 (Curtotti, Tuynman & Dylewski 2022). In 2022–23 the value of prawn production is forecast to increase (Curtotti et al. 2023). Between 2023–24 and 2027–28, the value of Australian prawn production is projected to remain stable (Curtotti et al. 2023; Curtotti, Tuynman & Dylewski 2022). Most local production is expected to supply local markets (Curtotti et al. 2023; Curtotti, Tuynman & Dylewski 2022).

Currently prawn farming provides over 300 full time equivalent jobs. Based on a 2018 report this was projected to increase to 1,200 direct and 3,000 indirect regional jobs within the next 5 years (Australian Prawn Farmers Association 2021). The same report forecasts product volumes to increase to 20,000 tonnes in the same period (Australian Prawn Farmers Association 2021).

In Queensland, the main prawn aquaculture state, during 2019–20 there were 18 producing prawn farms, down two from the previous financial year. Production in the prawn sector was 6,245 tonnes, valued at AU$124.6 million (State of Queensland 2021). Hatchery sales of prawns for 2019–20 were worth AU$1 million and 392 million postlarvae were produced (State of Queensland 2021). During 2019–20 in New South Wales, 494 tonnes of *P. monodon* was produced, valued at AU$9.89 million (Gippel 2021).

Other than prawns, crustacean species farmed in Australia include yabby (*Cherax destructor*), marron (*Cherax cainii* and *Cherax tenuimanus*) and redclaw crayfish (*Cherax quadricarinatus*), with production values of around AU$0.5, AU$2.1 and AU$0.9 million in 2020–21, respectively (Tuynman & Dylewski 2022). While all decapod crustaceans are susceptible to WSSV, other prawn hazards are also able to infect these species. For example, *C. quadricarinatus* is susceptible to infection with decapod iridescent virus 1 (Xu et al. 2016) and Taura syndrome virus has been found in *C. tenuimanus* (Biosecurity Australia 2006).

1.3.2 Fisheries

Wild-caught prawns were valued at AU$236 million (volume 16,258 tonnes) in 2020–21 (Tuynman & Dylewski 2022). The main target species for prawn fisheries in Australia are shown in Table 3 (Mobsby & Curtotti 2020; Steven, Dylewski & Curtotti 2021).

Table 3 Main target species for prawn fisheries in Australia

Species name	Common name(s)
Penaeus monodon	black tiger prawn, jumbo tiger, giant prawn, ghost prawn
Penaeus merguiensis	banana prawn, white banana prawn, Gulf banana prawn
Penaeus esculentus	brown tiger prawn, tiger prawn, common tiger prawn, southern tiger prawn
Penaeus semisulcatus	grooved tiger prawn, northern tiger prawn, green tiger prawn, green prawn
Penaeus indicus	red-legged banana prawn, banana prawn, Indian prawn, Indian white prawn, Tugela prawn, white prawn
Penaeus japonicus	Kuruma prawn, flower prawn, Japanese king prawn, Japanese prawn
Metapenaeus endeavouri	blue endeavour prawn, endeavour prawn, true endeavour prawn, brown prawn
Metapenaeus ensis	red endeavour prawn, offshore greasyback prawn, greasyback prawn, greentail prawn, false endeavour prawn
Metapenaeus macleayi	school prawn, eastern school prawn, white river prawn
Metapenaeus bennettae	greasyback prawn, inshore greasy back prawn, bay prawn, greentail prawn, river prawn
Penaeus latisulcatus	western king prawn, blue leg prawn, blue-legged king prawn, furrowed prawn, king prawn, brown prawn
Melicertus plebejus	eastern king prawn, king prawn, sand prawn
Penaeus longistylus	red-spot king prawn, red-spotted king prawn
Metapenaeopsis crassissima	scout velvet shrimp, coral prawn

Species name	Common name(s)
Macrobrachium rosenbergii (*Macrobrachium spinipes*)	giant freshwater prawn, giant river prawn, cherabin
Macrobrachium australiense	freshwater prawn, common Australian river prawn

Source: (Mobsby & Curtotti 2020; Steven, Dylewski & Curtotti 2021).

Other commercially important crustacean fishery species include marine lobsters and crabs. In 2020–21, rock lobster contributed 29.1% (AU$403 million) to the wild-caught gross value of fishery production (Tuynman & Dylewski 2022). Crabs are also one of the major species groups harvested from inshore and coastal Australian waters and were valued at AU$54 million in 2020–21 (Tuynman & Dylewski 2022).

1.4 Australia's trade in crustaceans

1.4.1 Prawn imports

Australia imports a significant quantity of prawns to meet domestic demand. These imports are generally of a lower unit value than prawns produced domestically (Curtotti, Tuynman & Dylewski 2022; Mobsby et al. 2021). A rise in global prices for prawns during late-2021 resulted in domestically produced prawns becoming more competitive in the domestic market (Curtotti, Tuynman & Dylewski 2022). The value of imported prawns for the 2020-2021 was $422 million (Tuynman & Dylewski 2022).

Following implementation of the enhanced import conditions in July 2017 there was a substantial decrease in the volume of uncooked prawns imported into Australia. The volume of uncooked prawns imported has recover to near 2016 levels, as shown in Table 4.

Table 4 Volume of prawns and prawn products imported into Australia across 2016–2022 calendar years

	volume (tonnes) imported per year						
Commodity	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Cooked [a]	9,588	18,504	17,432	14,154	14,182	14,196	13,977
Uncooked (raw) prawns	17,125	5,335 [b]	9,197	9,799	9,188 [c]	10,238	16,573
Breaded, battered and crumbed	1,875	5,849	3,141 [d]	2,685	2,437	2,242	3,545
Dumpling and dim sum-type product	774	1,277	956	571	459	952	1,603
Other prawn products [e]	2,395	1,560	537	990	1,687	2,006	2,416
Total	**31,757**	**32,525**	**31,263**	**28,199**	**27,953**	**29,634**	**38,114**

a Includes cooked breaded, battered and crumbed prawns and cooked dumpling and dim sum-type products. **b** Import of uncooked prawns was suspended 9 January – 6 July 2017. **c** Import conditions for deveining of uncooked prawns implemented in July 2020. **d** Import conditions for breaded, battered and crumbed prawns implemented in September 2018. **e** Other prawn products refers to all shelf stable prawn products including dried shrimp, prawn crackers and shelf-stable prawn paste. Source: Department of Agriculture, Fisheries and Forestry, Biosecurity Analytics Centre database of prawn imports.

1.4.2 Australian prawns processed overseas and imported into Australia

Whole, uncooked prawns are exported overseas for processing and then imported back into Australia. These prawns must meet the same conditions as prawns which originate from overseas. That is, they have had the head and shell removed, been deveined and tested negative pre-export and on-arrival for white spot syndrome virus (WSSV) and yellow head virus genotype 1 (YHV1). Currently, the only exception is uncooked Australian-origin prawns exported to Thailand and processed in the Thai Union Frozen Products Public Company Ltd., which is the only premise approved by both the department and Thailand's competent authority (CA) for this pathway. Prawns which are exported through this pathway are tested on-arrival for WSSV and YHV1 under a compliance-based intervention scheme.

Approximately 108 tonnes of uncooked Australian prawns were exported overseas for processing and re-imported into Australia during the 2022 calendar year (source: Department of Agriculture, Fisheries and Forestry, Agriculture Import Management System (AIMS)).

1.4.3 Crustacean exports

Global demand for prawns significantly declined during 2020–21 driven by COVID-19 based disruptions in the global travel, food service and accommodation sectors. Australia produces a relatively low volume of crustaceans, but our exports comprise several high unit value crustacean species focused on markets that were significantly impacted by COVID-19 and sporadic commodity-focused trade issues, including rock lobsters (Mobsby et al. 2021). The recovery after COVID-19 based disruptions brought a strong improvement in global prawn prices in late-2021 and early-2022. This rise in global prices for prawns led to higher unit export returns for Australian prawn exporters (Curtotti, Tuynman & Dylewski 2022). Prices are expected to moderate in 2022–23 (Curtotti et al. 2023).

In 2020–21, Australia exported more than 4,000 tonnes of prawns (from both aquaculture and wild fisheries sectors) valued at AU$73 million (Tuynman & Dylewski 2022). The major prawn export destinations for Australia in 2020–21 were Hong Kong (1,207 tonnes valued at AU$22.6 million), Japan (685 tonnes valued at AU$16.2 million) and Vietnam (1,017 tonnes valued at AU$15.5 million) (Tuynman & Dylewski 2022).

1.5 White spot disease outbreak in Australia

WSD was confirmed in a prawn farm on the Logan River in South-East Queensland on 1 December 2016. An emergency response led by the Queensland Government was initiated to contain and eradicate the disease. By February 2017, seven prawn farms were infected. Following a fallow period, some farms along the Logan River returned to production in late October 2018, with enhanced biosecurity controls in place.

The department's investigation into the cause of the initial outbreak focused on several possible entry pathways. The pathways considered were that the virus was introduced:

- via uncooked imported prawns being used as bait
- via imported aquatic feed or feed supplements
- through diseased broodstock or their progeny
- via a human element, including the importation of associated equipment; or

- that the virus was present in Australia, potentially in the environment at very low levels, but had not been detected previously.

Third parties have come to various conclusions about the possible sources of the outbreak. For example, the Australian Prawn Farmers Association, through the Fisheries Research and Development Corporation, commissioned Dr Ben Diggles to investigate the source of the outbreak, and he concluded that retail prawns being used as bait were the most likely source of the infection (Diggles 2017). The department's investigations have not determined the pathway of the 2016 incursion and other research suggests that the source of the virus may not have been imported prawns. For example, a study carried out in conjunction with the Queensland Department of Agriculture and Fisheries suggests the possibility that the outbreak was caused by a latent Australian virus (Oakey et al. 2019).

WSSV was again detected in prawns on two farms in the Logan River region in April 2020. These farms were also affected during the 2016–17 outbreak. At the time the virus was detected in 2020, the harvest on these farms was nearing completion. They underwent decontamination and laid fallow. A research project conducted in 2020 found some non-commercial crustacean species sampled from near Logan River farms and Moreton Bay within the Movement Regulated Area (MRA) tested positive for WSSV (Diggles 2020), which corresponded with the timing of the detection of WSSV on the two farms. The sites in Moreton Bay had previously tested positive for WSSV in March 2018. The results added value to the surveillance information but were not unexpected and did not indicate spread of WSSV outside the MRA.

The detection of WSSV in wild crabs and prawns in March 2020, and the re-emergence of the disease in nearby farmed prawns soon after suggested that WSSV had persisted at low levels in local species (Barrett et al. 2020). Genetic evidence supported this view as the virus strain was determined to be the same one that caused the original outbreak in 2016–17 (Australian Centre for Disease Preparedness 2021). Five Logan River farms commenced the production season in September 2020 and there have been no reports of WSD to date. Biosecurity Queensland is working with all prawn farms on the Logan River to ensure on-farm biosecurity management is appropriate.

As of February 2021, WSSV is considered established in populations of wild crustaceans within the Queensland MRA. This decision was made by a committee dealing with national aquatic animal disease emergency response, the Aquatic Consultative Committee for Emergency Animal Diseases (Aquatic CCEAD), based on surveillance and genetic evidence accumulated since 2017.

The Aquatic CCEAD was stood down from the WSD response given the transition from emergency response to ongoing containment and management. Responsibility for national management of WSD had been transferred to the Animal Health Committee.

The Aquatic CCEAD was reconvened on 23 August 2022 in response to a detection of WSSV in northern New South Wales (NSW) on 18 August 2022. Prawn broodstock held in a biosecure facility tested positive for WSSV and all (400) animals were destroyed, and the facility decontaminated. This incident of WSD was quickly contained and eradicated. After intensive investigation there was no conclusive evidence to determine the origin of this detection.

On 12 February 2023, WSD was again detected at a prawn farm in the Clarence Valley, NSW north coast. NSW Department of Primary Industries (DPI) was notified immediately, and the infected farm

was issued with formal biosecurity directions under the NSW *Biosecurity Act 2015* to implement strict biosecurity requirements. WSSV has since been detected in three farms in the area. At the time of preparing this report, there is no indication that the virus has spread beyond the affected premises. Genetic analysis using whole genome sequencing undertaken by the Australian Centre for Disease Preparedness (ACDP) confirmed the WSSV strains at the three farms are strongly similar to the WSSV detected in NSW in August 2022, and is a different lineage to the Queensland strain detected between 2016 and 2020. Investigations and tracing are underway to determine the origin of the infection. In the meantime, Australian states and territories have, or are considering, implementing precautionary enhanced import conditions to restrict the import of crustaceans and/or prawns for bait purposes collected from NSW waters.

1.5.1 Internal movement controls

Movement controls were first put in place by the Queensland Government on Logan River farms at the time of the WSD outbreak in December 2016 and were expanded to the MRA in March 2017 when WSSV was detected in northern Moreton Bay. A movement control order continues to be enforced for high-risk animals such as prawns, yabbies and marine worms, extending from Caloundra to the NSW border and west to Ipswich. Prawns intended for human consumption must be cooked or susceptible bait species must be irradiated prior to movement out of the MRA. These requirements or biosecurity measures are to prevent further spread of WSSV that has already been detected in the environment. They were implemented at the discretion of the Queensland Government and considered factors such as feasibility, enforceability, compliance and cost.

On 12 February 2021, the Aquatic CCEAD considered whether the risk management measures at the MRA remained appropriate given the transition from an emergency response to ongoing management of WSSV. Since no feasible alternatives were identified by the committee, the biosecurity measures remain in place but they agreed that alternative proposals could be considered on a case-by-case basis.

On 16 February 2023, and following the detection of WSSV on the NSW north coast, the NSW government issued a temporary movement control order which restricts the movement of raw, uncooked prawns and other decapod crustaceans out of the Clarence River. This control order was extended to minimise WSD risks in the Clarence River estuary and remained in place at the time of the release of this report. The control order supports risk management, surveillance and tracing activities to inform future management approaches.

1.5.2 Economic impact of the white spot disease outbreak

The economic impact of the WSD outbreak is ongoing in industries that operate out of movement control zones in place in Queensland and New South Wales.

Following the WSD outbreak in South-East Queensland, the Australian Government provided AU$1.87 million in 2016–17 as emergency funding for the industry to immediately help control the spread of WSD. During 2016–17, the Queensland Government spent more than AU$17 million on the operational response to WSD and committed a further AU$9 million over the two years to 2018–19. These costs were associated with the destruction of prawns from the diseased farm ponds, extra staffing levels, schemes of direct financial support for affected farms, surveillance and testing for WSSV in the Logan River and Moreton Bay, and education and awareness campaigns about WSSV. The production losses of the Logan River prawn farming industry in 2016–17 was approximately

AU$23.5 million (excluding their response costs) and it was estimated that the cost of lost hatchery and breeding stocks to be approximately AU$5–6 million (Ridge Partners 2017).

Between December 2016 and April 2017, the economic impact of the movement control order on the gross value of production of commercial wild fishery (including, beachworms, bloodworms, yabbies, mud and blue swimmer crabs, and prawns) was estimated as AU$20.5 million (Ridge Partners 2017). There was a severe impact on prawns and bloodworms which were destined for distribution as bait as these accounted for up to 80% of the Australian market (Commonwealth of Australia 2017). These costs are ongoing as the MRA remains in place.

The Australian and Queensland governments reimbursed or will reimburse AU$21.5 million and pledged a further AU$30 million for concessional loans. Some prawn farms also incurred the cost of installing additional infrastructure to prevent future production losses (for example, water filtration, pond lining, barriers for carrier exclusion.). The cost to implement extra biosecurity measures on an Australian prawn farm such as bird and crab netting, drum filtering and ozonation of water is estimated to be at least $1 million (Rosenberry 2017). Other estimates have put the cost to farms of establishing new biosecurity infrastructure to be approximately $87,600 per production pond hectare (Stephens 2017).

Further, the Australian Government provided financial support so those affected farms could remain destocked for 18 months. Prawn farmers will need to repay up to AU$4 million through an industry levy. The Australian Government also provided a AU$5 million assistance package to the Moreton Bay fishery industry, who continue to be affected by the movement restrictions related to the WSD outbreak.

The economic impact of the detection of WSSV in northern NSW in August 2022 and early-2023, including production losses and the impacts of the movement restrictions, was not available at the time of release of this report.

Other state governments involved in response or surveillance also incurred substantial costs. In addition, there is a national surveillance program to confirm Australia's health status with respect to WSSV freedom, the cost of which is borne by all Australian states and territories and the federal government. The surveillance program meets international standards to demonstrate freedom from WSD over a minimum two-year period.

It was estimated that the 6 month suspension in uncooked prawn imports cost AU$383 million to Australian businesses, with resulting price rises for consumers (Seafood Importers Association of Australasia 2017). The import suspension also resulted in the loss of several thousand tonnes of seafood product that would otherwise have been sold in Australia (Seafood Importers Association of Australasia 2017).

1.5.3 Inspector-General of Biosecurity review

Following the WSD outbreak and the suspension of uncooked prawn imports in January 2017, the Inspector-General of Biosecurity (IGB) undertook a review of the effectiveness of biosecurity controls implemented by the department for this commodity. When considering the likelihood of each pathway being the source of infection, the IGB report stated:

> I consider that the use of infected imported prawns for bait or berley in the Logan River was a possible pathway of infection for 1IP [first infected premises]. Nevertheless, all the possible pathways of WSD entry into Australia, and their risk mitigation, need active consideration by all relevant stakeholders (Inspector-General of Biosecurity 2017).

The report, published in December 2017, contained 22 recommendations. Twenty are complete and the department is close to finalising the remaining 2 (see Appendix C) (Inspector-General of Biosecurity 2017).

IGB report on the implementation of the recommendations

In 2019, the IGB reported on the implementation of the 22 recommendations made in the IGB's review in 2017. The report stated that major progress was made in implementing the recommendations, and that the implemented measures substantially increased compliance (Inspector-General of Biosecurity 2019). These included pre-border and border measures such as seals-intact inspections and laboratory testing of samples of all incoming prawn consignments, which led to a far more compliant trade, and a better understanding by industry and governments in the exporting countries of how to meet Australia's requirements. The IGB also found that testing of retail prawns showed that the new measures had greatly reduced the risk of imported prawn products bringing in diseases of biosecurity concern. Lastly, the report commented that the prawn review was underway and that outcomes of it could support the significant gains made in managing the biosecurity risks of prawns and prawn products since 2017 (Inspector-General of Biosecurity 2019).

1.6 Use of prawns as bait and berley by recreational fishers in Australia

The Prawn IRA 2009 considered that the regular introduction of imported prawns, intended for human consumption, into the aquatic environment through use as bait or berley presented a significant pathway for exposure of wild crustaceans to imported prawns potentially infected with hazards. Surveys conducted by Kewagama Research in 2002 and 2007 investigating the use of prawns, intended for human consumption, as bait or berley provided significant data inputs for the exposure assessment and when considering biosecurity measures in the Prawn IRA 2009.

The Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) and the University of Canberra conducted the *National recreational fishing survey* during 2019–20. The survey collected data on participation, motivation, annual expenditure, and regional economic flows of recreational fishing, as well as the use of prawns for bait and berley by recreational fishers.

The department expected to have preliminary data from the *2019–20 National recreational fishing survey*, as it relates to the use of prawns as bait and berley in early-2020, for consideration and inclusion when preparing the *Review of the biosecurity risks of imported prawns*–draft report. However, the catastrophic bushfires experienced over the 2019–20 summer period and the COVID-19 pandemic significantly impacted participants in the surveys and the data was not available. Therefore, the department released the draft report using assumptions based on available data.

ABARES fast-tracked assessment of the bait and berley portion of the *2019–20 National recreational fishing survey* (2019–20 Bait and berley survey) so that the outcomes could be considered in this final

report. Section 1.6.1 Summary of the outcomes of the 2019–20 Bait and berley survey provides an overview of the outcomes of the 2019–20 Bait and berley survey. The survey outcomes and the way in which they affected previous conclusions presented in the draft report have been reviewed and changes in assumptions presented.

Results were reported nationally, and by jurisdiction. When considering the data presented in the 2019–20 Bait and berley survey, the department used the national results. Applying separate consideration of each jurisdiction is not appropriate given this risk review applies to import of prawns into Australia, and not into individual jurisdictions. Additionally, recreational fishers may travel to other jurisdictions to fish, using the same behaviours in those other locations.

ABARES will release the standalone 2019–20 Bait and berley survey report and the parent survey *2019–20 National recreational fishing survey* on the department's website in due course.

1.6.1 Summary of the outcomes of the 2019–20 Bait and berley survey

Method

The *2019–20 National recreational fishing survey* was conducted in three stages, each with a specific role in the broader goal of measuring the social and economic contribution of recreational fishing, including direct and indirect expenditure:

- Stage 1 was a population wide screening survey conducted at the end of 2018.
- Stage 2 was an online survey of Australian fishers and non-fishers conducted between September 2019 and May 2020.
  - Participants in stage 2 of the survey were recruited via a range of methods deliberately biased towards avid recreational fishers.
  - Questions on bait and berley use in the previous 12 month period from the date each respondent completed the survey were nested into this stage
- Stage 3 included an 18-month diary phase (December 2019 to June 2021) and a wash-up survey.

Model-based weighting was used to correct for biases in responses. To weight the results, first, known characteristics of Australia's recreational fishers were identified and used to specify a 'superpopulation'. Statistical weighting was then used to develop raked weights that corrected for biases in the responses received compared to this superpopulation. A detailed description of the methods used in the survey is provided in Moore et al. (2022). It is noted that using weighted frequency data for further analyses or comparison should be done with caution due to the difficulties in extrapolating from samples to the population, despite weighting procedures.

In brief, the *2019–20 National recreational fishing survey* participants were recruited using multiple methods designed to ensure that all types of fishers had the opportunity to participate in the survey. The sampling method was designed to achieve a representative sample of all types of recreational fishers across Australia, and to deliberately over-sample avid fishers.

Survey design and questions

From the 23,747 participants in stage 2:

- 17,596 responded to questions regarding their fishing participation.
- A subset of 11,849 participants indicated they fished within the last 12 months.

- Of the 11,849 participants who fished in the last 12 months, a further subset of 5,514 recreational fishers indicated they had used any type of bait in the previous 12 months.
  - These participants were all asked if they had seen advice regarding the use of imported seafood prawns as bait, even if they had not used prawns as bait in the past 12 months.
- Of the 5,514 who had used bait in the past 12 months, a subset of 3,396 indicated they had used prawns.
- Of the 3,396 participants who used prawns in the past 12 months, 822 reported using prawns purchased from a seafood retailer rather than a bait supplier.
- The 822 recreational fishers who reported using prawns purchased from a seafood retailer as bait, were also asked about:
  - the prawn (product) type and volume range used
  - the origin (local or imported)
  - reasons for purchasing prawns from a seafood retailer rather than a bait supplier.

Results from the bait and berley questions

Types of bait used

The 2019–20 Bait and berley survey asked the 5,514 recreational fishers who indicated they had used any type of bait in the previous 12 months, what type of bait they had used. 3,396 indicated they had used prawns. Nationally, the top five bait types in order of usage, were prawns (42.5%), other saltwater fish (38.7%), cephalopods (34.8%), saltwater worms (24.4%), and other shellfish (18.9%) (Moore et al. 2022).

Use of prawns intended for human consumption as bait or berley for recreational fishing

The respondents who had used prawns as bait in the past 12 months were asked whether they had purchased prawns from a bait shop or a seafood retailer. 822 indicated they had purchased prawns from a seafood retailer. As a weighted sample size, this was ~20% of the respondents who had used prawns as bait in the past 12 months. As a proportion of total fishers who had fished in the past 12 months (the population considered by the department when assessing risks associated with imported prawns being used as bait), this represents a much smaller proportion (822/11,849).

Prawn product type used as bait

The 2019–20 Bait and berley survey did not report or estimate total volumes of prawns used as fishing bait. However, it did gather information about the individual usage ranges of different product types, as summarised in Table 5. The proportions are of fishers who reported using prawns purchased from a seafood retailer, not of the total fishers who fished in the past 12 months.

Uncooked whole prawns were the most common prawn type purchased from a seafood retailer for use as bait (82%) (Moore et al. 2022). It is assumed these prawns are Australian origin given the import conditions for uncooked, whole prawns in place at the time of the survey.

Of the respondents who purchased prawns for bait from a seafood retailer, 40% reported using uncooked but shelled prawns. This demonstrates that this form of prawn is still attractive for use as bait but that whole uncooked prawns are the preferred form.

The 2019–20 Bait and berley survey found that cooked prawns, both shelled and not shelled were used as bait at a rate similar to uncooked but shelled prawns. With 41% of respondents who purchased prawns as bait from a seafood retailer, indicating they had used cooked but not shelled prawns and 32% saying they had used cooked and shelled prawns for bait use.

Processed prawns were the least common product type used. With 24% of respondents reporting use of processed prawns (for example, skewers, marinated, breaded or battered, part of a dumpling, spring roll or other product, or butterflied). It is important to note that for the purposes of the 2019–20 Bait and berley survey questions, examples of processed prawns included skewered, butterflied and marinated prawns, which the department considers as uncooked (shelled) prawns in the context of Australia's import conditions. Therefore, the usage of breaded, battered and crumbed and dumpling-type products as bait is likely less than what is reported in these usage patterns for processed prawns.

Table 5 Summary of volume ranges and proportion of prawn product types purchased from a seafood retailer and used as bait or berley (Moore et al. 2022).

Product type	Usage rates (%)		
	>1 kg	<1 kg	None
Uncooked (whole)	24	58	18
Uncooked (shelled)	6	34	60
Cooked (whole)	14	27	60
Cooked (shelled)	10	22	68
Processed [a]	6	18	77

[a] processed (for example, skewers, marinated, breaded or battered, part of a dumpling, spring roll or other product, or butterflied).

Imported or Australian-origin prawn usage

The 2019–20 Bait and berley survey asked respondents that used prawns as bait sourced from a seafood retailer whether they had used Australian or imported prawns. Most respondents (78%) said they either often used Australian-origin prawns or sometimes used Australian-origin prawns (45.8% and 32.7%, respectively) (Moore et al. 2022). Conversely, 33.5% of respondents said they either often used imported prawns or sometimes used imported prawns (9.4% and 24.1%, respectively) (Moore et al. 2022).

Awareness of not using imported seafood prawns as bait

In the 2019–20 Bait and berley survey, all respondents, regardless of whether they used prawns as bait or not, were asked if they had seen advice regarding the use of imported seafood prawns as bait. Nationally, 47% of respondents who answered this question said that they had seen advice on not using imported prawns as bait (Moore et al. 2022). The jurisdiction with the highest proportion of respondents who were aware of this advice was Queensland (68.2%), likely reflecting the education and awareness programs in that region at the time (Moore et al. 2022).

Why recreational fishers use prawns intended for human consumption as bait or berley for recreational fishing

The 2019–20 Bait and berley survey asked respondents to select out of 10 categories their reasons ('often a reason', 'sometimes a reason' or 'not a reason') for obtaining prawns from a seafood retailer, rather than from a bait supplier. The top 4 'often a reason' options selected nationally were:

- better quality than bait prawns (36%)
- fresher than bait prawns (34%)
- cheaper than bait prawns (26%)

- easier to obtain than bait prawns (17%) (Moore et al. 2022).

1.6.2 Conclusions about the 2019–20 Bait and berley survey

When considering the 2019–20 Bait and berley survey outcomes, the department made conclusions and assumptions and applied them to the risk assessments in this final report. These are:

- The department considers the total fishers who fished in the past 12 months (11,849) to be the population of interest in considering likelihood and usage proportions of prawns intended for human consumption being used as bait or berley. This is because this group represents the behaviour of recreational fishers as a population.
- Prawns were the most common bait-type used by recreational fishers.
- Most fishers prefer Australian-origin prawns for use as bait or berley.
- A small proportion of fishers who fished in the past 12 months purchased prawns from a seafood retailer for use as bait or berley. Of the prawns purchased from the seafood retailer:
  - Whole, uncooked prawns are the preferred prawn product type used as bait or berley.
  - Cooked (whole or shelled) prawns are used as bait or berley at a rate similar to uncooked, shelled prawns.
  - Processed prawns are the product type least likely to be used for bait or berley.
- There are varying reasons for why recreational fishers source prawns from a seafood retailer, but the primary motives focus on the quality and availability of the prawns.

Sections 4.3.3 Key considerations of the exposure assessments and Appendix D Likelihood and amount of imported prawns (or associated wastes) entering the general environment of the exposure groups provide further detail on the 2019–20 Bait and berley survey outcomes and on the way the department has considered these results in the risk assessments in this final report.

2 Method

This chapter provides a high-level summary of the method used by the department when conducting risk reviews.

The World Organisation for Animal Health (WOAH, formerly OIE) *Aquatic animal health code* (WOAH Code) describes 'General obligations related to certification' in chapter 5.1 (WOAH 2022c).

The WOAH Code states in Article 5.1.2. (WOAH 2022c) that:

> The import requirements included in the international aquatic animal health certificate should assure that commodities introduced into the importing country comply with WOAH standards. Importing countries should align their requirements with the recommendations in the relevant standards of the WOAH. If there are no such recommendations or if the country chooses a level of protection requiring measures more stringent than the standards of the WOAH, these should be based on an import risk analysis conducted in accordance with chapter 2.1.

Article 5.1.2 (WOAH 2022c) further states that:

> The international aquatic animal health certificate should not include measures against pathogenic agents or diseases that are not WOAH listed, unless the importing country has demonstrated through an import risk analysis, carried out in accordance with Section 2, that the pathogenic agent or disease poses a significant risk to the importing country.

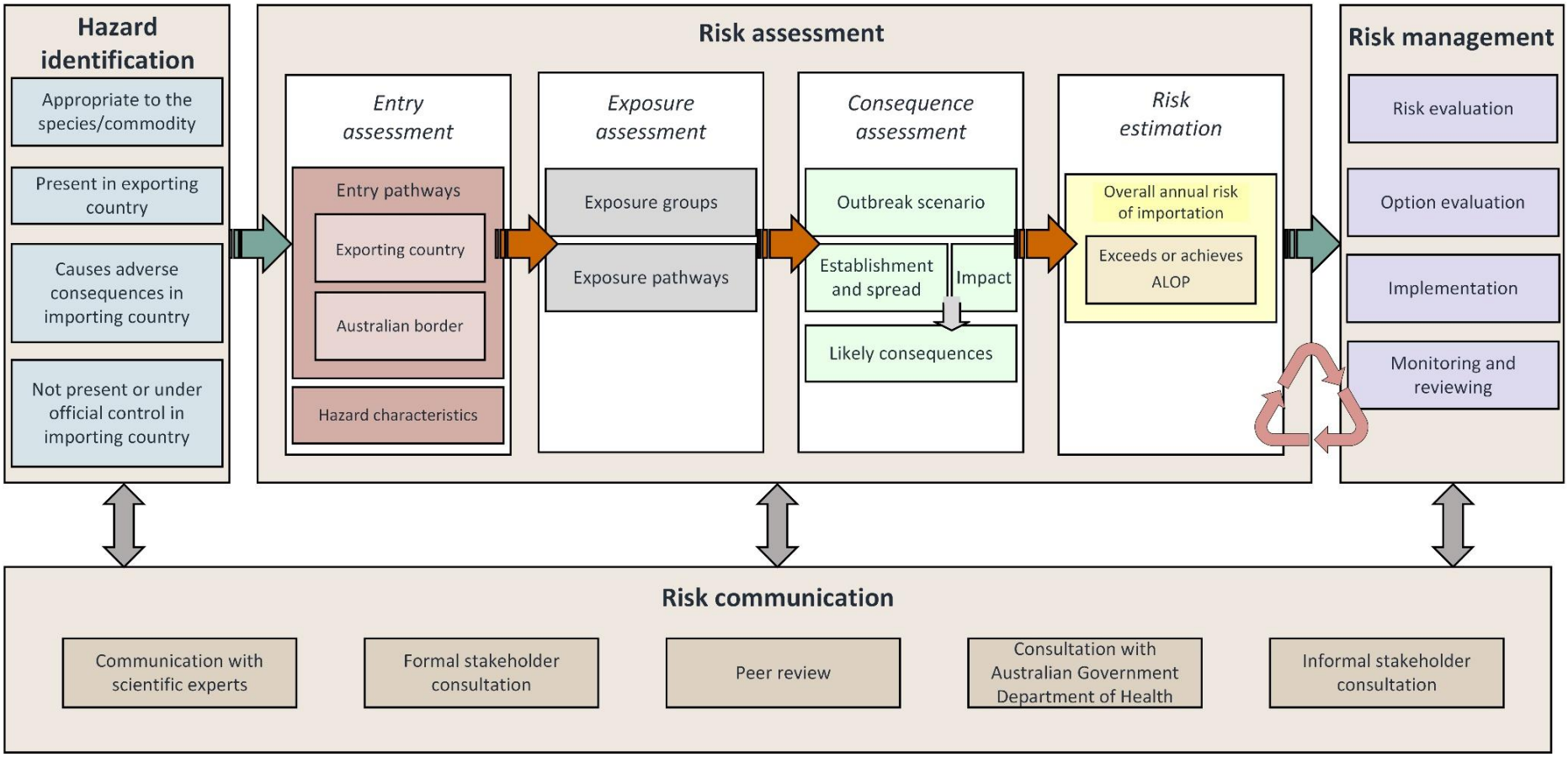
The four components of risk analysis as described in chapter 2.1. (WOAH 2022c) of the WOAH Code are:

- hazard identification
- risk assessment (entry, exposure and consequence assessments and risk estimation)
- risk management
- risk communication.

Hazard identification, risk assessment and risk management are sequential steps within a risk analysis. Risk communication is an ongoing process and includes both formal and informal consultation with stakeholders.

Figure 2 shows the components of the import risk analysis process and the steps within the risk assessment process.

Figure 2 Components of the import risk analysis process



The import risk analysis process comprises of four components. **Hazard identification** the first component involves identifying the pathogenic agents associated with the importation of a commodity, that could potentially produce adverse consequences. **Risk assessment** the second component, is completed for each hazard. It estimates the risks associated with the hazard and is comprised of four steps. **Entry assessment** describes the pathway(s) for the introduction of the hazard into Australia, and estimates the likelihood of entry for that hazard. **Exposure assessment** determines, for each hazard, the likelihood that a susceptible species in Australia would be exposed to the agent via imported prawns or associated wastes. **Consequence assessment** describes the potential consequences of a given exposure, and estimates the probability of them occurring. **Risk estimation** integrates likelihood of entry and exposure and likely consequences to obtain the overall annual risk associated with a hazard. **Risk management** the third component of risk analysis is the process of identifying, selecting and implementing

measures that can be applied to reduce the level of risk of a hazard, while at the same time, ensuring that negative effects on trade are minimised. **Risk communication** the fourth component of risk analysis, is an ongoing process and includes both formal and informal consultation with stakeholders and peer review.

2.1 Risk review

Risk review is not defined or described in the WOAH Code, however risk analysts recognise risk review as an essential component of the risk analysis process (Barry 2007; FSA 2006; Purdy 2010).

Australia applies a process of risk review to the biosecurity risks associated with the importation of an animal commodity (animal product or live animal) for which current biosecurity measures exist.

Sources of information drawn on for this risk review include (this list is not exhaustive):

- the World Organisation for Animal Health (WOAH, formerly OIE) *Aquatic animal health code* (WOAH 2022c)
- the World Organisation for Animal Health (WOAH, formerly OIE) *Manual of diagnostic tests for aquatic animals* (WOAH 2022l)
- *Generic import risk analysis report for prawns and prawn products 2009* (Prawn IRA 2009) (Biosecurity Australia 2009)
- current requirements for importation of prawns into Australia
- relevant scientific information, including research commissioned by the department and conducted by the University of Arizona.
- expert opinion
- the *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* report (Moore et al. 2022)
- results of the department's monitoring of imported prawns for emerging diseases program.
- policies adopted by other countries for the importation of prawns.

Risk, defined by the WOAH Code as 'the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health' (WOAH 2022c), is dynamic in nature and changes with time. Consequently, regular review of risk should be undertaken.

Risk review differs from the monitoring and review component of risk management, as described in the WOAH Code, in that each component of the risk analysis process (hazard identification, risk assessment and risk management) is reviewed under the risk review process. Based on updated scientific information, if it is identified that there has been a change (either an increase or a decrease) in the biosecurity risk associated with a live animal or animal products currently imported into Australia, biosecurity measures can be revised accordingly.

2.2 Review of hazard identification

The WOAH Code (Article 2.1.2) describes hazard identification as a classification step done to identify potential hazards that may be associated with the importation of a commodity (WOAH 2022c).

In accordance with the WOAH Code (WOAH 2022c), a pathogenic agent was considered a potential hazard relevant to the importation of prawns if it was assessed to be:

- 'appropriate' to the species to be imported, or from which the commodity is derived
- present in the exporting country

- able to potentially produce adverse consequences in the importing country
- not present in the importing country, and if present, associated with a listed disease, or subject to control or eradication measures.

Where evidence for the inclusion or exclusion of a pathogenic agent was equivocal, a judgement was made based on the strength of the available evidence to implicate prawns in disease transmission.

2.3 Review of risk assessment

A review of risk factors relevant to the entry, exposure and consequence assessment was conducted for each hazard retained for risk review. If definitive information on risk factors was not found through literature review or contact with relevant experts, any uncertainties were identified and documented.

Based on the information reviewed, a conclusion was reached for each hazard about whether a significant change in biosecurity risk had occurred that was relevant to the importation of prawns into Australia. Assumptions and judgements that were made in drawing conclusions for each hazard were documented in the relevant risk review chapters.

The likelihood that a hazard would enter an importing country, and the likelihood of exposure of susceptible animals to the hazard, were determined through an 'entry assessment' and 'exposure assessment', respectively. The 'likelihood of establishment and spread' and the 'adverse impacts', were determined through a 'consequence assessment'. The risk assessment for an identified hazard concluded with 'risk estimation'.

Chapter 4 further describes the method used to assess risk and summarises the general considerations taken into account when undertaking this risk review. Appendix D provides a detailed discussion of the general considerations.

2.4 Review of risk management

The WOAH Code (chapter 2.1) (WOAH 2022c) divides risk management into four components:

- risk evaluation
- option evaluation
- implementation
- monitoring and review.

2.4.1 Risk evaluation

Risk evaluation is the process of comparing the risk estimated in the risk assessment with the WOAH member's appropriate level of protection (ALOP).

Australia's ALOP has not changed since the Prawn IRA 2009 was published. Risk evaluation during this risk review was based on the conclusions drawn from the risk assessments conducted for each hazard. A judgement was made to determine whether risk management was warranted to achieve Australia's ALOP.

2.4.2 Option evaluation

Option evaluation ultimately results in selection of a biosecurity measure which will reduce the risk associated with the importation of a product to a level which achieves the WOAH member country's ALOP. The process of option evaluation includes considering the efficacy and feasibility of the biosecurity measure.

The efficacy is the degree to which an option reduces the likelihood and/or magnitude of adverse health and economic consequences. Evaluating the efficacy of the options selected is an iterative process that involves their incorporation into the risk assessment and then comparing the resulting level of risk with that considered acceptable. The evaluation for feasibility normally focuses on technical, operational and economic factors affecting implementation of the risk management options.

In this risk review, detailed consideration of numerous biosecurity measures for imported prawns was undertaken and documented (see chapter 5 Options for biosecurity management of imported prawns).

2.4.3 Implementation

Implementation is the process of following through with the risk management decision and ensuring that the biosecurity measures are in place.

2.4.4 Monitoring and review

Monitoring and review are the ongoing processes by which biosecurity measures are continually audited. This ensures that they are achieving the results intended.

The department is responsible for monitoring and reviewing any applied biosecurity measures to enable the safe importation of prawns.

2.5 Risk communication

Risk communication is defined in the WOAH Code as:

> the interactive transmission and exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions among risk assessors, risk managers, risk communicators, the general public and other interested parties (WOAH 2022c).

In conducting risk analyses and policy reviews, the department consults with the Department of Health to ensure that public health considerations are included in the development of Australia's animal biosecurity policies. Consultation with external stakeholders is a standard procedure for all import risk analyses and risk reviews. Consultation on this risk review enables stakeholder feedback on draft conclusions and recommendations about Australia's biosecurity policies. Peer review is an essential component of risk communication to obtain a scientific critique and to ensure that the data, information, methods and assumptions are the best available.

When undertaking this risk review, the department put in place the Prawn Review Liaison Officer (PRLO) who has been the first point of contact for all related questions. The PRLO has provided

periodic updates on this risk review since it began. The PRLO will remain in place until at least the release of the final report.

3 Hazard identification

For this review, the list of pathogenic agents (potential hazards) of potential biosecurity concern was compiled from:

- diseases listed by the World Organisation for Animal Health (WOAH, formerly OIE) as affecting prawns (and other species where relevant) (OIE 2021c)
- diseases identified in the *Generic import risk analysis report for prawns and prawn products 2009* (Prawn IRA 2009)
- other diseases (including emerging) identified as occurring in prawns.

The hazard identification process is described in section 2.2 Review of hazard identification.

Table 6 shows the list of potential hazards identified through this review and summarises the results of the hazard identification process, including the reason for removal or retention of each pathogenic agent. The worldwide distribution of non-WOAH listed potential hazards is drawn from that reported in the literature. The worldwide distribution of WOAH listed potential hazards has been collected using WOAH-WAHIS (WOAH World Animal Health Information System) and other literature and information. Where an assessment of disease freedom is to be made by the department, it is based on evidence provided by the country of export.

Many pathogenic agents are ubiquitous and may already be present in Australia. Others are emerging, opportunistic, or are of uncertain relevance in prawns due to limited or insufficient information. All pathogenic agents of prawns were considered potential hazards when compiling the list. However, a potential hazard could only be considered a hazard if it met the criteria outlined in section 2.2 Review of hazard identification.

The pathogenic agents identified as hazards and retained for risk review are listed at the end of this chapter (see section 3.2 Pathogenic agents retained for risk review).

3.1 Emerging diseases

The WOAH defines an emerging disease as:

> A disease, other than listed diseases, which has a significant impact on aquatic animal or public health resulting from a change of known pathogenic agent or its spread to a new geographic area or species; or a newly recognised or suspected pathogenic agent.

The department has ongoing media and scientific literature feeds about biosecurity issues for all animal species, those feeds are reviewed by technical experts. Information about emerging diseases that are reported would likely be picked up through these information sources. Under the WOAH, Member Countries are required to notify of the occurrence of listed diseases and emerging disease events. Member Countries are also encouraged to provide the WOAH with other important aquatic animal health information.

Various information sources are used by the department to monitor emerging and existing pathogenic agents of prawns that may present a biosecurity risk to Australia. These include but are not limited to:

- aquatic animal disease experts
- departmental data on trade patterns
- departmental data on prawn testing (for example, random batch testing)
- grey literature (for example, media reports)
- Network of Aquaculture Centres in Asia Pacific (NACA)
- World Organisation for Animal Health (WOAH, formerly OIE)
- other countries
- scientific conferences, webinars and workshops
- scientific literature.

Information sources are constantly being reviewed by technical experts and if concerning information arises, the department will review the risk of the pathogenic agent and determine if it achieves Australia's appropriate level of protection (ALOP). If the risk exceeds Australia's ALOP then the department can take immediate action. It is important to note that these reviews are most often not a formal risk review, and therefore they are only released publicly if the review determines that changes need to be made. For example, the import conditions for prawns were strengthened in September 2018 and July 2020 in response to new information about trade trends and biosecurity risks.

The difficulty with emerging diseases is that there is often limited information available on which to assess the biosecurity risk. The department bases its decisions on biosecurity risk on the available science. Where evidence is lacking, a judgement is made based on the strength of the available information. This is done in a conservative way, assuming that the information available is accurate and represents the significance of the situation.

Table 6 Hazard identification and refinement

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
Viruses	–	–	–	–	–	–	–	–
Aquabirnaviruses	Farmed and wild finfish species Isolated from molluscs	No	Yes	Some species	Americas Asia Europe	No	**No:** there is little evidence to indicate that aquabirnaviruses would be associated with imported prawns. An infectious pancreatic necrosis virus (IPNV)-related aquabirnavirus is present in Australia, but it has not been reported in prawns. There are a small number of historical reports of the isolation of an IPNV-like virus from prawns, however the sporadicity of the reports suggest infection (if it occurs) is very rare. Additionally, there was insufficient evidence in those reports to conclude that the isolated viruses were responsible for disease and there is no conclusive evidence that prawns are susceptible (even experimentally) to IPNV or IPNV-related aquabirnaviruses.	(Biosecurity Australia 2009; Bovo et al. 1984; Crane et al. 2000; Dobos 1995; Mortensen 1993)
Bacilliform virus of *Crangon crangon* Includes *Crangon crangon* nudivirus (CcNV) and Gammanudivirus cracrangoni.	*Crangon crangon*	No	Uncertain	No	Belgium United Kingdom	No	**No**: there is little evidence to indicate that bacilliform virus of *Crangon crangon* would be associated with imported prawns. There is no evidence that native Australian crustacean species are susceptible to this virus. The department will continue to monitor the situation with respect	(Bateman et al. 2021; Bateman & Stentiford 2017; Van Eynde et al. 2018)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							to bacilliform virus of *Crangon crangon*.	
Baculoviral midgut gland necrosis virus (BMNV) Includes other BMNV-like viruses.	*Penaeus japonicus* (*Penaeus chinensis, Penaeus monodon, Penaeus semisulcatus* (experimental infection only))	No	No	No	Japan and Republic of Korea (BMNV) East and South-East Asia (BMNV-like viral infections)	No	**No**: BMNV is no longer of international significance in prawn health due to improved biosecurity and production practices. BMNV is no longer WOAH-listed. BMNV is not included in Australia's *National list of reportable diseases of aquatic animals*. It is considered that BMNV-associated clinical disease in imported prawns would be rare due to the life stages more commonly affected and that adverse consequences would not result as BMNV is readily controllable (for example, by the routine washing of eggs and nauplii in clean seawater).	(Biosecurity Australia 2009; Lightner 1996a, 2004; Momoyama & Sano 1996; Rajendran, Makesh & Karunasagar 2012; Sano et al. 1981)
Baculovirus penaei (BP) (tetrahedral baculovirosis)	Various penaeid species, including: *Penaeus aztecus* *Penaeus duorarum* *Penaeus marginatus* *Penaeus stylirostris* *Penaeus vannamei*	No	No	No	Americas Hawaii	Yes	**No**: BP is no longer of international significance in prawn health due to improved biosecurity and production practices. BP is no longer WOAH-listed. BP is not included on Australia's *National list of reportable diseases of aquatic animals*. It is considered that BP-associated clinical disease in imported prawns would be rare due to the life stages more commonly affected, that the life stage of the prawn affects infectivity and that adverse consequences would not result as BP is readily controllable (for example, by the routine washing of	(Bateman & Stentiford 2017; Brock et al. 1986b; Couch 1974; Hammer, Stuck & Overstreet 1998; Overstreet 1994; Rubio Limonta & Silveira Coffigny 2012)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							eggs and nauplii in clean seawater) in the hatchery. There is no evidence that BP causes mortalities in the wild where Australia's only susceptible species is present.	
Bay of Piran shrimp virus	*Palaemon elegans*	No	No	No	Mediterranean Sea	No	**No**: not considered to cause significant disease.	(Bateman & Stentiford 2017; Vogt 1996)
Beihai shrimp virus genotypes 1 -6 Includes other Beihai-like viruses.	Various penaeid and caridean species including: *Exopalaemon carinicauda* *Metapenaeus* sp. *Penaeus vannamei*	No	Uncertain	Beihai picorna-like virus	China	No: disease agent not identified in Prawn IRA 2009	**No**: a Beihai like virus is present in Australia and not subject to control or eradication. Beihai shrimp virus is not listed by the WOAH. RNA-seq analysis of the transcriptome of prawn species discovered genome sequences of Beihai shrimp virus (genotypes 1 - 6). Beihai shrimp viruses have been detected in healthy prawns. The department will continue to monitor the situation with respect to Beihai shrimp viruses.	(Huerlimann et al. 2018; Shi et al. 2016)
Covert mortality nodavirus (CMNV) (viral covert mortality disease (VCMD)/ covert mortality disease (CMD) / 'bottom death' disease)	Various penaeid and caridean species including: *Exopalaemon carinicauda* *Macrobrachium rosenbergii* *Penaeus chinensis* *Penaeus japonicus* *Penaeus monodon* *Penaeus vannamei*	No	Yes	No	China Ecuador India Mexico Thailand Vietnam	No: disease agent not identified in Prawn IRA 2009	**Yes:** CMNV has caused serious losses in China and cumulative mortalities of up to 80–90% of *Penaeus vannamei* in culture. CMNV is not included on Australia's *National list of reportable diseases of aquatic animals.* CMNV is included in the *List of Diseases in the Asia-Pacific.* This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification*	(Flegel 2014; Huang 2015; NACA, OIE-RRAP & FAO 2018; Wang et al. 2018; Wang et al. 2021b; Wang et al. 2019; Xu et al. 2021a; Zhang et al. 2014; Zhang et al. 2018; Zhang et al. 2017b)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
	Various aquatic animals including: *Carassius auratus* *Danio rerio* *Mugilogobius abei* *Paralichthys olivaceus* *Larimicthys polyactis* *Apostichopus japonicas* *Corophium sinense* *Diogenes edwardsii* *Ocypode cordimundus* *Parathemisto gaudichaudi* *Tubuca arcuate*						and will be retained for risk assessment.	
***Crangon crangon* flavivirus (CcFV)** (Including Crangon crangon flavi-like viruses)	*Crangon crangon*	No	Uncertain	No	Belgium Germany	No: disease agent not identified in Prawn IRA 2009.	**No:** there is little evidence to indicate that CcFV would be associated with imported prawns. RNA-seq analysis of the transcriptome of randomly sampled *Crangon crangon* discovered putative genome sequences of CcFV. The department will continue to monitor the situation with respect to CcFV.	(Martínez-Alarcón et al. 2019; Parry & Asgari 2019; Van Eynde et al. 2020)
***Crangon crangon* novel viruses**	*Crangon crangon*	No	Uncertain	No	Belgium	No: disease agent not identified in Prawn IRA 2009.	**No**: there is little evidence to indicate that the *Crangon crangon novel viruses* would be associated with imported prawns. There is no evidence that native Australian crustacean species are susceptible to these viruses. Next generation sequencing of the virome of *Crangon crangon* discovered the 15 novel viruses.	(Van Eynde et al. 2020)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							Crangon crangon novel viruses have been detected in apparently healthy prawns. The department will continue to monitor the situation with respect to the *Crangon crangon novel viruses*.	
Crustacea hepe-like virus 1 (CHEV1)	*Macrobrachium rosenbergii*	No	Uncertain	No	China	No: disease agent not identified in Prawn IRA 2009.	**No**: disease is currently restricted to *M. rosenbergii* from China. There is little evidence to indicate that it would be associated with imported prawns and no evidence that prawn species other than *M. rosenbergii* are susceptible to infection. The department will continue to monitor the situation with respect to CHEV1.	(Dong et al. 2020b)
Decapod hepanhamaparvovirus 1 Previously known as decapod hepandensovirus 1, hepatopancreatic parvovirus (HPV) and *Penaeus monodon* densovirus (PmDNV). Includes multiple strains.	Various penaeid and caridean species including: *Macrobrachium rosenbergii* *Penaeus californiensis* *Penaeus chinensis* *Penaeus merguiensis* *Penaeus monodon* *Penaeus setiferus* *Penaeus stylirostris* *Penaeus vannamei*	No	Yes	Some strains	Africa Americas Asia Middle East	Yes	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. A genetically different isolate of DHPV infecting *P. vannamei* in Korean farms have been reported. Studies aimed at determining and analysing the complete genome are still to be published at the time of the release of this report. The department will continue to monitor the situation with respect to this isolate.	(La Fauce, Elliman & Owens 2007; Srisala et al. 2020b; Walsh et al. 2017)
Decapod iridescent virus 1 (DIV1) Includes *Cherax quadricarinatus* iridovirus (CQIV) and shrimp	Various penaeid and caridean species including: *Macrobrachium nipponense*	Yes	Yes	No	China Indian Ocean Taiwan	No: disease agent not identified	**Yes:** the department notes that although there is limited information regarding DIV1, it is considered a serious emerging disease in aquaculture — large	(Chen et al. 2019a; Chung 2020; Li, Xu & Yang 2017; NACA 2016; Qiu et al. 2017; Qiu et al. 2018a; Qiu

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
hemocyte iridescent virus (SHIV).	*Macrobrachium rosenbergii* *Penaeus japonicus* *Penaeus monodon* *Penaeus vannamei* Various aquatic animals including: *Cherax quadricarinatus* *Cladocera* (water flea) *Procambarus clarkii* (*Exopalaemon carinicauda, Pachygrapsus crassipes* and *Eriocheir sinensis* (experimental infection only))					in Prawn IRA 2009	volumes of imported prawns are sourced from areas that may be affected by DIV1. DIV1 is listed by the WOAH. DIV1 is included in the *List of diseases in the Asia-Pacific*, and Australia's *National list of reportable diseases of aquatic animals*. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	et al. 2018b; Qiu et al. 2019b; Ramsden & Smith 2018; Xu et al. 2016)
Decapod penstylhamaparvovirus 1 Previously known as decapod penstyldensovirus 1 and infectious hypodermal and haematopoietic necrosis virus (IHHNV). Includes multiple strains.	Various penaeid and caridean species including: *Macrobrachium rosenbergii* *Penaeus californiensis* *Penaeus monodon* *Penaeus setiferus* *Penaeus stylirostris* *Penaeus vannamei*	Yes	Yes	Yes, multiple strains.	Africa Asia Central America Middle East North America Pacific islands South America	No	**No:** present in Australia and is not subject to control or eradication. IHHNV is listed by the WOAH, is listed on the Australian *List of reportable diseases of aquatic animals* and the *List of diseases in the Asia-Pacific*.	(Arbon et al. 2021; Bateman & Stentiford 2017; Lightner et al. 1994; Lu et al. 1991; OIE 2019c; Owens et al. 1992; Rai et al. 2012; Srisala et al. 2020b)
***Farfantepenaeus duorarum* nodavirus (FdNV)**	*Peneaus duorarum*	No	Uncertain	No	Mexico	No: disease agent not identified in Prawn IRA 2009.	**No:** Random shotgun sequencing of the RNA virome of *P. duorarum* discovered genome sequences of FdNV. FdNV has been only detected in healthy prawns. It is unknown if	(Ng et al. 2013)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							FdNV has the potential to cause disease. The department will continue to monitor the situation with respect to FdNV.	
Infectious myonecrosis virus (IMNV)	Various penaeid species including: *Penaeus monodon* *Penaeus semisulcatus* *Penaeus vannamei* (*Artemia franciscana, Penaeus esculentus, Penaeus merguiensis, Penaeus stylirostris* and *Penaeus subtilis* (experimental infection only))	Yes	Yes	No	Brazil China Egypt India Indian Ocean Indonesia Malaysia Myanmar Republic of Korea	Yes	**Yes:** IMNV is WOAH listed and has been responsible for considerable losses in the Brazilian prawn farming industry and is present in Asia. IMNV is included on the Australian *List of reportable diseases of aquatic animals* and the *List of diseases in the Asia-Pacific*. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	(Aly et al. 2021; Coelho et al. 2009; Sahul Hameed et al. 2017; Tang et al. 2005)
Infectious precocity virus (IPV) (Infectious precocity virus disease, iron prawn syndrome (IPS)) Also known as *Crustaflavivirus infeprecoquis* gen. nov.	Various penaeid and caridean species including: *Macrobrachium rosenbergii* *Macrobrachium nipponense* *Procambarus clarkia* *Penaeus vannamei* *Penaeus monodon* Various aquatic animals including: *Oratosquilla oratoria* *Insect Anisops kuroiwae*	No	Uncertain	No	China	No: disease agent not identified in Prawn IRA 2009	**No:** disease has only been reported in China in farmed *M. rosenbergii* prawns. IPV causes sexual precocity with decreased larval development. There is little evidence to indicate that it would be associated with imported prawns. The department will continue to monitor the situation with respect to Infectious precocity virus.	(Dong et al. 2021; Wang et al. 2023; Zhao et al. 2023)
Irido-like virus / *Protrachypene precipua* iridovirus	*Protrachypene precipua*	No	No	No	Ecuador	No	**No:** not considered to cause significant disease.	(Lightner & Redman 1993)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
Laem Singh virus (LSNV) Includes Wenzhou shrimp virus genotype 9 (WZSV9) and an associated integrase-containing element (ICE) as component causes of monodon slow growth syndrome (MSGS)).	Various penaeid species including: *Metapenaeus dobsoni* *Penaeus merguiensis* *Penaeus monodon* *Penaeus vannamei*	No	Yes	No	China Egypt India Indonesia Malaysia Philippines Sri Lanka Thailand Vietnam	Yes (considered as MSGS): but there was insufficient information to conduct a risk assessment	**Yes**: MSGS is included on Australia's *National list of reportable diseases of aquatic animals.* It has been determined that LSNV and WZSV9 are different isolates of the same virus species. Although there is limited information regarding LSNV and its role in MSGS, large volumes of imported prawns are sourced from countries that may be affected by MSGS. LSNV will be considered in context with MSGS. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	(NACA 2016; Panphut et al. 2011; Poornima et al. 2012; Prakasha et al. 2007; Shi et al. 2016; Sittidilokratna et al. 2009b; Taengchaiyaphum et al. 2020)
Lymphoid organ vacuolization virus (LOVV)	*Penaeus stylirostris* *Penaeus vannamei*	No	No	No	Americas Hawaii	No	**No:** not considered to cause significant disease.	(Bonami et al. 1992; Lightner et al. 1992)
Lymphoid organ virus (LOV)	*Penaeus monodon*	No	No	Yes	Not reported	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Cowley et al. 2000)
Lymphoidal parvo-like virus (LPV)	Various penaeid species	No	No	Yes	Not reported	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens, De Beer & Smith 1991)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
Macrobrachium hepatopancreatic bidnavirus (MHBV)	*Macrobrachium rosenbergii*	No	Uncertain	No	Thailand	No: disease agent not identified in Prawn IRA 2009	**No:** disease is currently restricted to *M. rosenbergii* from Thailand. There is little evidence to indicate that it would be associated with imported prawns and no evidence that prawn species other than *M. rosenbergii* are susceptible to infection.	(Gangnonngiw et al. 2023)
***Macrobrachium nipponense* reovirus (MnRV)**	*Macrobrachium nipponense*	No	Uncertain	No	China	No: disease agent not identified in Prawn IRA 2009	**No**: disease is currently restricted to *M. nipponense* from China. There is little evidence to indicate that it would be associated with imported prawns and no evidence that prawn species other than *M. nipponense* are susceptible to infection. The department will continue to monitor the situation with respect to MnRV.	(NACA 2016; Zhang et al. 2016)
***Macrobrachium rosenbergii* Golda virus (MrGV)**	*Macrobrachium rosenbergii*	No	Uncertain	No	Bangladesh	No: disease agent not identified in Prawn IRA 2009	**No:** the disease is currently restricted to *M. rosenbergii* from Bangladesh. There is insufficient evidence to indicate that it would be associated with imported prawns due to it primarily affecting larval stages. The department will continue to monitor the situation with respect to MrGV.	(Hooper et al. 2020)
***Macrobrachium rosenbergii* Taihu virus (MrTV)** (disease of seven days)	*Macrobrachium rosenbergii*	No	Uncertain	No	China	No: disease agent not identified in Prawn IRA 2009	**No:** the disease is currently restricted to *M. rosenbergii* from China and there is no evidence of spread or reports since 2016. There is insufficient evidence to indicate that it would be associated with imported prawns due to it primarily affecting larval stages.	(NACA 2016; Pan et al. 2016)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							The department will continue to monitor the situation with respect to MrTV.	
Monodon baculovirus (MBV) Also known as singly enveloped nuclear polyhedrosis virus from *Penaeus monodon* (PmSNPV). Includes plebejus baculovirus bennettae baculovirus and spherical baculovirus (*Penaeus monodon* nudivirus (PmNV).	Various penaeid and caridean species, including: *Macrobrachium rosenbergii* *Penaeus esculentus* *Penaeus indicus* *Penaeus kerathurus* *Penaeus merguiensis* *Penaeus monodon* *Penaeus penicillatus,* *Penaeus semisulcatus*	No	No	Some strains	Americas Asia East Africa Madagascar Mediterranean (Italy) Middle East Indo Pacific region	No	**No:** some strains present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Bateman & Stentiford 2017; Biosecurity Australia 2009; Lightner & Redman 1981; Rajendran, Makesh & Karunasagar 2012; Vickers, Webb & Young 2000)
Mourilyan virus (MoV) Includes Wenzhou shrimp virus 1 (WZSV1).	Various penaeid species including: *Penaeus japonicus* *Penaeus monodon*	No	No	Yes	China Fiji Malaysia Thailand Vietnam	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. WZSV1 is thought to be a strain of MoV.	(Li et al. 2015; Oanh et al. 2011; OIE 2007) (Jeff Cowley [CSIRO Agriculture & Food] 2018, pers. comm., 22 October)
***Pandalus montagui* bacilliform virus (PmBV)**	*Pandalus montagui*	No	No	No	United Kingdom	No: disease agent not identified in Prawn IRA 2009	**No**: not considered to cause significant disease, is restricted to the United Kingdom and there is little evidence to indicate that PmBV would be associated with imported prawns.	(Bateman, Hicks & Stentiford 2007; Bateman & Stentiford 2017)
Penaeid haemocytic rod-shaped virus (PHRV)	Hybrid *Penaeus esculentus* × *Penaeus monodon*	No	No	Yes	Not reported	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic*	(Owens 1993a)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							animals and is not subject to control or eradication.	
***Penaeus monodon* metallodensovirus (PmMDV)**	*Penaeus monodon*	No	Uncertain	No	Vietnam	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease. The department will continue to monitor the situation with respect PmMDV.	(Pénzes et al. 2020)
***Penaeus vannamei* nodavirus (PvNV)** (white tail disease-like muscle necrosis)	*Penaeus vannamei* (*Penaeus monodon* (experimental infection only))	No	Uncertain	No	Belize	No: disease agent not identified in Prawn IRA 2009	**No**: not considered to cause significant disease. The department will continue to monitor the situation with respect to PvNV.	(Tang et al. 2007b; Tang et al. 2011)
Reo-III and IV viruses Also known as Penaeid shrimp Reo-like virus, Reo-like virus in *Penaeus vannamei.* Includes Reo-like virus and Palaemon B-cell reo-like virus.	Various penaeid species	No	No	No	Americas Asia Europe	No	**No**: not considered to cause significant disease.	(Krol, Hawkins & Overstreet 1990; Nash et al. 1988)
Rhabdovirus of penaeid shrimp (RPS)	*Penaeus stylirostris* *Penaeus vannamei*	No	No	No	Ecuador Hawaii	No	**No:** there is little evidence to indicate that RPS would be associated with imported prawns as there is no evidence that RPS cause disease in adult prawns. Subadult/adult prawns have been experimentally infected with no mortalities or clinical signs of disease. Infection and mortalities by RPS have only been reported in experimentally infected postlarvae and juveniles. RPS has been reported to be closely related to Carp sprivivirus.	(Biosecurity Australia 2009; Lightner 1996a; Lu et al. 1991) (Johnson et al. 1999; Lu & Loh 1994a; Lu & Loh 1992; Lu & Loh 1994b; Lu, Loh & Nadala 1994; Nadala et al. 1992a; Nadala et al. 1992b)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							The department will continue to monitor the situation with respect to RPS.	
Sergestid iridovirus (SIV)	*Acetes erythraeus*	No	No	No	Madagascar	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease and there is little evidence to indicate that SIV would be associated with imported prawns.	(Tang et al. 2007a)
Spawner-isolated mortality virus (SMV)	*Cherax quadricarinatus* *Penaeus monodon*	No	Yes	Yes	Philippines	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens & McElnea 2000)
Taura syndrome virus (TSV)	Various penaeid species, including: *Penaeus aztecus* *Penaeus ensis* *Penaeus monodon* *Penaeus setiferus* *Penaeus stylirostris* *Penaeus vannamei* (*Penaeus chinensis, Penaeus merguiensis* and *Macrobrachium rosenbergii*	Yes	Yes	No	Americas China East Africa Hawaii Indonesia Malaysia Middle East Myanmar Philippines Republic of Korea Taiwan Thailand	Yes	**Yes:** TSV is WOAH Listed and is associated with significant losses in prawn farming environment and is widespread throughout the world. TSV is included on the Australian *List of reportable diseases of aquatic animals*, and the *List of diseases in the Asia-Pacific*. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	(Brock 1997b; Jimenez et al. 2000; Lightner 1996a)
Wenzhou Shrimp virus (WZSV) genotypes 2–8 and 10 WZSV8 includes *Penaeus vannamei* picornavirus (PvPV) and *Penaeus*	Various penaeid and caridean species including: *Exopalaemon carinicauda* *Metapenaeus* sp. *Penaeus monodon*	No	No	Some strains present (WZSV1, 2 and 8)	China Americas Indo-Pacific	No: disease agent not identified in Prawn IRA 2009	**No**: some strains present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. WZSV is not listed by the WOAH.	(Huerlimann et al. 2018; Li et al. 2015; Shi et al. 2016; Taengchaiyaphum et al. 2020) (Arbon et al. 2021) (Cruz-Flores et al. 2022; Liu et al.

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
vannamei solinvivirus (PvSV). Excluding WZSV1 (refer Mourilyan virus) and WZSV9 (refer Laem Singh virus).	*Penaeus vannamei* *Solenocera crassicornis*						RNA-seq analysis of the transcriptome of prawn species discovered genome sequences of WZSV genotypes 1–10. PvPV and PvSV are thought to be a strain of WZSV8. WZSV have been detected in healthy prawns. The department will continue to monitor the situation with respect to exotic strains of WZSV.	2021; Srisala et al. 2022) (Jeff Cowley [CSIRO Agriculture & Food] 2018, pers. comm., 22 October)
White spot syndrome virus (WSSV)	All decapod (order Decapoda) crustaceans from marine, brackish or freshwater sources challenged with infection with WSSV are susceptible	Yes	Yes	Limited to south-east Queensland and under official control program.	Americas Asia East Africa Middle East	Yes	**Yes:** WSSV is WOAH listed and associated with significant losses in prawn farming environment and is widespread throughout the world. WSSV is included on the Australian *List of reportable diseases of aquatic animals*, and the *List of diseases in the Asia-Pacific*. Australia is managing an outbreak of WSSV which is limited to south-east Queensland and is under official control and eradication program with surveillance activities ongoing. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	(Inouye et al. 1994; Lightner et al. 1998; Takahashi et al. 1994; van Hulten et al. 2001; Wongteerasupaya et al. 1996; Yang et al. 2001)
White tail disease (WTD) Also known as *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and extra small virus (XSV) / *Macrobrachium* muscle virus (MMV).	Various penaeid and caridean species including: *Macrobrachium rosenbergii* *Penaeus indicus* *Penaeus japonicus*	Yes	No	Yes	China Dominican Republic French West Indies India Indonesia	No	**No:** present in Australia and not subject to control or eradication. WTD is included on the Australian *List of reportable diseases of aquatic animals* and the *List of diseases in the Asia-Pacific*.	(Gangnonngiw et al. 2020; Kibenge & Godoy 2016; Murwantoko et al. 2016; Pillai & Bonami 2012; Ravi et al. 2009; Senapin et al. 2012;

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
	Penaeus monodon *Penaeus vannamei*				Malaysia Myanmar Taiwan Thailand Vietnam			Sudhakaran et al. 2006)
Yellow head virus genotype 1 (YHV1)	Various penaeid species including: *Penaeus monodon* *Penaeus stylirostris* *Penaeus vannamei* (*Palaemonetes pugio, Metapenaeus affinis* (experimental infection only))	Yes	Yes	No	Indonesia Malaysia Mexico Myanmar Philippines Sri Lanka Taiwan Thailand	Yes	**Yes:** YHV1 is WOAH listed and has been found in many commercially important wild and cultured species throughout the world at relatively high prevalence and is increasingly being associated with co-infections and stunted growth. Infection with YHV1 is included on the Australian *List of reportable diseases of aquatic animals*, and the *List of diseases in the Asia-Pacific*. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	(Chayaburakul et al. 2004; Flegel et al. 2004)
Yellow head virus genotypes 2–7 (YHV2–YHV7)	Various penaeid species	No	No	Some strains present (YHV2, YHV6 and YHV7)	China India Indonesia Malaysia Mozambique Philippines Taiwan Thailand Vietnam	Yes: listed as YHV	**No:** some strains present in Australia and not subject to control or eradication. YHV2–YHV7 are not listed by the WOAH. Infection with gill associated virus (YHV2) is included on Australia's *National list of reportable diseases of aquatic animals.* Infection with YHV2–YHV7 are not included in the *List of diseases in the Asia-Pacific*.	(Arbon et al. 2021; Chen et al. 2018; Cowley et al. 2000; Cowley et al. 2015; Liu et al. 2014; Mohr et al. 2015; Munro, Callinan & Owens 2011; NACA, OIE-RRAP & FAO 2020b; OIE 2017a; Walker et al. 2001; Wijegoonawardane et al. 2008)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							YHV2, YHV6 and YHV7 are present in Australia. The susceptibility of *P. monodon* and *P. merguiensis* to YHV7 is being determined by the Australian Centre for Disease Preparedness (formerly Australian Animal Health Laboratory). YHV3–YHV5 have been detected in healthy prawns around the world and are rarely or never associated with disease. The department will continue to monitor the situation with respect to YHV3–YHV5.	(Jeff Cowley [CSIRO Agriculture & Food] 2018, pers. comm., 13 November)
Yellow head virus genotype 8 (YHV8)	Various penaeid and caridean species including: *Macrobrachium rosenbergii* *Penaeus chinensis* *Penaeus japonicus* *Penaeus vannamei*	No	Uncertain	No	China Republic of Korea	No: disease agent not identified in Prawn IRA 2009	**Yes:** YHV8 is not WOAH listed. Infection with YHV8 is not included in the *List of diseases in the Asia-Pacific*. However, YHV8 is currently responsible for disease outbreaks in China. Large volumes of prawns are exported from China to Australia. It is unknown if Australian prawns are susceptible to YHV8. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	(Kim et al. 2020; Thitamadee et al. 2016; Zhu et al. 2016)
Yellow head virus genotypes 9 and 10 (YHV9 and YHV10)	*Penaeus vannamei*	No	No	No	Not reported	No: disease agent not identified in Prawn IRA 2009	**No:** *P. monodon* and *P. merguiensis* are not susceptible to YHV9 and YHV10. YHV9 and YHV10 have been detected from imported prawns in Australia by Australian Centre for	(Cowley et al. 2015; FRDC 2018) (FRDC Conference abstract, Moody et al. 2019)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							Disease Preparedness (formerly Australian Animal Health Laboratory). but it is unknown if these genotypes were associated with disease in the source populations. YHV9 and YHV10 are not WOAH listed or subject to control or eradication in Australia and their worldwide distribution is unknown. YHV9 and YHV10 are not included on the *List of diseases in the Asia-Pacific*. The department will continue to monitor the situation with respect to YHV9 and YHV10.	
Chlamydia, mycoplasma, rickettsia, spiroplasma	–	–	–	–	–	–	–	–
***Chlamydia* species**	Various aquatic animals, including penaeid species	No	No	Yes	Ecuador	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Jimenez et al. 2001; Owens & Hall-Mendelin 1990)
***Mycoplasma* species**	Various aquatic animals, including penaeid species	No	No	Yes	China	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Ghadersohi & Owens 1999; Yang 1997)
Planctomycete bacteria	*Penaeus monodon*	No	No	Yes	Not reported	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Fuerst et al. 1997)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
***Rickettsia*-like organisms (RLOs)**	Various penaeid and caridean species including: *Macrobrachium rosenbergii* *Pandalus platyceros* *Penaeus marginatus* *Penaeus merguiensis* *Penaeus monodon* *Penaeus stylirostris*	No	No	Some present	Canada Hawaii Madagascar Mexico South-East Asia	Yes: considered under necrotising hepatopan creatitis bacterium	**No:** there is little evidence of significant disease associated with RLOs in prawns and little information to indicate that RLOs would be associated with imported prawns. The department will continue to monitor the situation with respect to RLOs.	(Anderson et al. 1987; Bower, Meyer & Boutillier 1996; Brock et al. 1986a; Nunan et al. 2003a; Nunan et al. 2003b; Pillai & Bonami 2012; Wang et al. 2001)
Rickettsia "*Candidatus* Hepatobacter penaei" Previously known as necrotising hepatopancreatitis bacterium (NHPB). (necrotising hepatopancreatitis (NHP))	Various penaeid species including: *Penaeus aztecus* *Penaeus duorarum* *Penaeus marginatus* *Penaeus merguiensis* *Penaeus setiferus* *Penaeus stylirostris* *Penaeus vannamei* (*Penaeus monodon* (experimental infection only)) (*Homarus americanus* (pathogen specific PCR result but no active infection))	Yes	Yes	No	Americas Malaysia Thailand United States of America Vietnam	Yes	**Yes:** *"Candidatus* Hepatobacter penaei*"* is WOAH listed and associated with significant losses in prawn farming environment and is widespread throughout the world. *"Candidatus* Hepatobacter penaei*"* is included on the Australian *List of reportable diseases of aquatic animals*, and the *List of diseases in the Asia-Pacific*. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	(Nunan et al. 2013)
***Rickettsia*-like bacteria (RLB) associated with milky haemolymph disease** Caused by 4 isolates of *Rickettsia*-like bacteria. (milky haemolymph syndrome, milky	*Carcinus maenas* *Panulirus* species *Penaeus monodon*	No	Yes	No	East Africa France Madagascar Mozambique Tanzania	No: disease agent not identified in Prawn IRA 2009	**No**: there is little evidence to indicate that rickettsia-like bacterium of milky disease would be associated with imported prawns. There are limited reports of rickettsia-like bacterium of milky	(Australian Government Department of Agriculture Fisheries and Forestry 2012; Lightner et al. 2012b; Nunan et al. 2003a;

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
haemolymph disease (MHD))	(*Penaeus vannamei* (experimental infection only))				United Kingdom Vietnam		disease infection in cultured prawns, including one report of disease in cultured *Penaeus monodon* and *Penaues vannamei* that has only been infected with MHD experimentally. The department will continue to monitor the situation with respect to rickettsia-like bacterium of milky disease.	Nunan et al. 2003b; Nunan et al. 2010)
***Spiroplasma* species** Includes *Spiroplasma eriocheiris.*	*Macrobrachium nipponensis* *Macrobrachium rosenbergii* *Penaeus vannamei* *Eriocheir sinensis* *Procambarus clarkii*	No	Yes	No	China Colombia	No: disease agent not identified in Prawn IRA 2009	**No**: associated with disease in crabs and crayfish, but limited reports of *Spiroplasma* species infecting penaeid and *Macrobrachium* species. *Spiroplasma eriocheiris* infection is included in the *List of Diseases in the Asia-Pacific*. The department will continue to monitor the situation with respect to *Spiroplasma* species.	(Liang et al. 2011; NACA, OIE-RRAP & FAO 2018; Nunan et al. 2005; Nunan et al. 2004; Srisala et al. 2018; Wang et al. 2011)
Bacteria	–	–	–	–	–	–	–	–
***Aerococcus viridans* var homari (gaffkemia)**	Various aquatic animals, including: *Penaeus aztecus* *Pandalus platyceros* *Homarus species* Several crab species	No	Yes	No	Europe North America United Kingdom	No	**No**: there is little evidence to indicate that *Aerococcus viridans* var *homari* would be associated with imported prawns.	(Stebbing et al. 2012)
***Aeromonas* species**	Various aquatic animals, including penaeid and caridean species such as:	No	No	Some species present (*Aeromonas salmonicida* subsp.	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Pillai & Bonami 2012; Yuan et al. 2021)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
	Macrobrachium rosenbergii *Procambarus clarkii*			*salmonicida* is exotic)			(Note: *Aeromonas salmonicida* subsp. *salmonicida* is not associated with prawns)	
Aquatic epicommensal bacteria Includes *Cytophaga* specie, *Flavobacterium* species, *Leucothrix mucor, leucothrix* species and *Thiothrix* species.	Various aquatic species, including penaeid and caridean species	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Lewis, Leong & Mock 1982)
Bacillus cereus (white patch disease)	*Penaeus vannamei*	No	No	Yes	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Velmurugan et al. 2015)
Bacillus licheniformis	*Penaeus vannamei*	No	No	Yes	Colombia Presumed to be widely distributed.	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Galvez et al. 2016; Prada-Peñaranda et al. 2018)
Citrobacter freundii (Water bubble disease)	Various terrestrial and aquatic animals including freshwater and marine crustaceans, fish, amphibians, and birds *Macrobrachium rosenbergii* *Procambarus clarkii*	No	No	Yes	China Presumed to be widely distributed.	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. *Citrobacter freundii* is an opportunistic bacterium. A strain of *C. freundii* (GDZQ201912) has been reported causing water bubble disease with mortalities in farmed *M. rosenbergii* in China.	(Osei Sekyere & Reta 2021; Zhao et al. 2022)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
***Diplococcus* species**	Various aquatic animals, including penaeid species	No	No	Yes	Presumed to be widely distributed.	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens & Hall-Mendelin 1990)
Enterobacter cloacae	Various aquatic animals including penaeid and caridean species such as: *Macrobrachium rosenbergii* *Procambarus clarkii*	No	No	Yes	Widely distributed.	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. *E. cloacae* has been reported causing infections in China, where it has been associated with disease in farmed *P. clarkii*, and with slow growth and poor survival rates of *M. rosenbergii* in hatcheries. *E. cloacae* has also been reported causing mortality in *Mugil cephalus*, in India.	(Dong et al. 2020a; Gao et al. 2020a; Gao et al. 2019; Gao et al. 2020b; Sekar et al. 2008)
***Enterococcus* species** Including *Enterococcus faecium*	*Macrobrachium rosenbergii*	No	No	Some species present, including *E. faecium*	Taiwan	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Chen et al. 2003; Cheng & Chen 1998a, b; Pillai & Bonami 2012)
***Flavobacterium* species**	Various aquatic animals including penaeid and caridean species such as: *Macrobrachium rosenbergii* *Penaeus stylirostris*	No	No	Some species present	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Escobedo-Bonilla 2016; Lightner 1985; Sheu et al. 2011; Uddin et al. 1998)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
***Flexibacter* species**	Various aquatic animals, including penaeid and caridean species	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Mourino et al. 2008)
Hepatopancreatic brush border lysis (HBL) bacterium	*Palaemon elegans*	No	No	Yes	Adriatic Sea	No	**No**: not considered to cause significant disease.	(Vogt 1997; Vogt & Strus 1998)
Infection associated with *Aeromonas schubertii*, *Proteous penneri* and *Vibrio* species (V. *alginolyticus, V. harveyi* and *V. parahaemolyticus*) (red body disease)	Various penaeid species including: *Penaeus monodon* *Penaeus stylirostris* *Penaeus vannamei*	No	No	Some species present	Americas Asia Hawaii	No: disease agent not identified in Prawn IRA 2009	**No:** some species present in Australia, not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. Red body disease has also been reported to be a generalized syndrome, with more than one cause.	(Alapide-Tendencia & Dureza 1997; Cao et al. 2014; Cao et al. 2015; Lightner & Redman 1985)
***Lactococcus* species** Including *L. garvieae* and *L. lactis* (white muscle disease (WMD))	*Macrobrachium rosenbergii*	No	No	Some species present	Taiwan	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Chen et al. 2001; Wang et al. 2008)
***Micrococcus* species**	Various species, including penaeids	No	No	Yes	China India Pakistan Singapore Sri Lanka	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Lalitha & Surendran 2004)
***Mycobacterium* species**	Various aquatic animals, including	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic*	(Brock, Nakagawa & Shimojo 1986;

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
	penaeid and caridean species						*animals* and is not subject to control or eradication.	Lightner & Redman 1986)
***Photobacterium* species** Includes *Ph. damselae subsp. damselae and Ph. Phosphoreum*.	Various aquatic animals including fish, mollusc, penaeid and caridean species	No	No	Yes	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Liu, Liu & Li 2016; Prayitno & Latchford 1995; Rivas, Lemos & Osorio 2013; Singaravel et al. 2020; Vaseeharan et al. 2007)
***Providencia* species** Includes *P. rettgeri* and *P. alcalifaciens*.	Various peneaid species including: *Penaeus vannamei* Associated with infection in a wide range of hosts including crocodiles. Some species are associated with opportunistic infection in humans.	No	No	Yes	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No**: present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. *Providencia* species are not on the *Australian national notifiable diseases* or the *Non-national notifiable diseases in Australia's States and Territories* lists for human health.	(Benedict & Shilton 2016; Cao et al. 2018b; Department of Health 2018b, a; Gai et al. 2017)
***Pseudomonas* species**	Various aquatic animals, including penaeid and caridean species such as: *Macrobrachium rosenbergii*	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Pillai & Bonami 2012)
***Sherwanella* algae**	Various aquatic animals including: *Penaeus vannamei* Known human pathogen	No	No	Yes	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. *Sherwanella algae* is not on the *Australian national notifiable diseases* or the *Non-national*	(Cao et al. 2018a; Department of Health 2018b, a)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							notifiable diseases in Australia's States and Territories lists for human health.	
***Spirillum* species**	Various penaeid species	No	No	Some species present	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Escobedo-Bonilla 2016)
***Staphylococcus* species**	*Macrobrachium rosenbergii*	No	No	Some species present	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Vijayan et al. 2005a)
***Streptococcus* species**	*Penaeus monodon* *Penaeus vannamei*	No	No	Some species present	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	Lightner 2009, Hasson et al. 2009, (cited in (Lightner et al. 2012b))
Vibrio brasiliensis	Various aquatic animals including: *Artemia* *Oncorhynchus mykiss* *Penaeus vannamei*	No	Unknown	No	Brazil China Malaysia	No: disease agent not identified in Prawn IRA 2009	**No:** *V. brasiliensis* is described as associated with experimental mortalities in *P. vannamei* and *O. mykiss*. There is no evidence that prawn species other than *P. vannamei* are susceptible to infection and it is unknown if infection can occur through natural exposure. The department will continue to monitor the situation with respect to *V. brasiliensis*.	(Li et al. 2021)
***Vibrio parahaemolyticus* strains causing glass post-larvae**	*Penaeus vannamei*	No	Uncertain	No	China	No: disease agent not identified	**No:** *V. parahaemolyticus* strains causing glass post-larvae have been associated with mortalities in 5 to 12 days old *P. vannamei* postlarvae	(Harkell 2020a, c; Huang 2020; Yang et

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
Also known as highly lethal *V. parahaemolyticus* strains and *V. parahaemolyticus* strains Vp-JS20200428004–2 (Glass post-larvae, translucent post-larvae disease, highly lethal *Vibrio* disease)						in Prawn IRA 2009	in Chinese hatcheries. Disease by these strains has been referred to 'glass post-larvae' as postlarvae become translucent before dying. Glass post-larvae was first thought to be caused by Hepatopancreas and digestive tract necrosis virus (HINV), but following studies demonstrated that the disease was less likely caused by a virus and identified *V. parahaemolyticus* strains as the causal agent (called highly lethal *V. parahaemolyticus* strains and *V. parahaemolyticus* strain Vp-JS20200428004–2). There is little evidence to indicate that *V. parahaemolyticus* strains causing glass post-larvae would be associated with imported prawns as there is insufficient evidence that they cause disease in adult prawns. The department will continue to monitor the situation with respect to Vibrio parahaemolyticus strains causing glass post-larvae.	al. 2022; Zhang 2021; Zou et al. 2020)
***Vibrio parahaemolyticus* strains containing Pir toxins (Vp AHPND)** (acute hepatopancreatic necrosis disease (AHPND))	Various penaeid species including: *Penaeus chinensis* *Penaeus japonicus* *Penaeus monodon* *Penaeus semisulcatus* *Penaeus vannamei*	Yes	Yes	No. (Australia has reported hepatopancreatitis in prawns to the WOAH but it did not satisfy the WOAH case definition of AHPND as it was caused by a *Vibrio harveyi* clade and	Bangladesh China Costa Rica Egypt Japan Malaysia Mexico Myanmar Peru Philippines	No: disease agent not identified in Prawn IRA 2009	**Yes**: AHPND is listed by the WOAH and is associated with significant losses in prawn farming environment and is widespread in countries likely to export large quantities of prawns to Australia. AHPND is also included on the Australian *List of reportable diseases of aquatic animals*, and the *List of diseases in the Asia-Pacific.* AHPND means infection with strains of *V. parahaemolyticus* (Vp_{AHPND}) that contain a ~70-kbp	(Dong et al. 2017b; Eshik et al. 2018; Flegel 2012; Kondo et al. 2015; Lee et al. 2015; Lightner et al. 2012a; Megahed 2018; NACA, OIE-RRAP & FAO 2016; OIE 2016a, 2017b, 2019a; Tran et al. 2013b)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
				Vp AHPND was excluded.)	Republic of Korea Taiwan Thailand Vietnam		plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB. Although there are reports of the isolation of other *Vibrio* species from clinical cases of AHPND, only Vp_{AHPND} has been demonstrated to cause AHPND. The department will continue to monitor the situation with respect to other species of *Vibrio* which may cause AHPND. This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	
Vibrio penaeicida	Various ornamental crustacean species. *Penaeus japonicus* *Penaeus stylirostris* (*Penaeus vannamei* and *Penaeus indicus* (experimental infection only))	No	Yes	Yes	Japan New Caledonia Republic of Korea	Yes	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. *Vibrio penaeicida* has been isolated from southern rock lobster, tropical rock lobster and striped trumpeter in Australia.	(Aguirre-Guzman, Ascencio & Saulnier 2005; Avarre et al. 2003; Carson et al. 2009; Choi et al. 2018; Costa et al. 1998a; Costa et al. 1998b; Costa et al. 1996; de la Peña, Naka & Muroga 1998; Goarant et al. 1998; Takahashi, Shimoyama & Momoyama 1985) (Jeremy Carson [Tasmania, Department Primary Industries Parks Water &

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
								Environment] 2018, pers. comm., 24 July)
***Vibrio* species** Includes species associated with luminous Vibriosis such as *Vibrio harveyi*. Excludes Vp AHPND.	Various aquatic animals including penaeid and caridean species	No	No	Some species present	Widely distributed	No	**No:** other than the Vp_{AHPND}, *Vibrio* species affecting penaeid and caridean species are either not considered to cause significant disease, or are present in Australia, are not included on the Australian *List of reportable diseases of aquatic animals* and are not subject to control or eradication.	(Harris & Owens 1999; OIE 2017b) Owens et al. 1992 cited in (Owens, Austin & Austin 1996)
Fungi	–	–	–	–	–	–	–	–
***Achlya* species**	Various aquatic animals including freshwater and marine crustaceans	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Meyers 1990)
***Aphanomyces* species** Excludes A*. astaci* and *A. invadans*.	Various aquatic animals including: *Daphnia magna* Copepods Freshwater crayfish *Macrobrachium rosenbergii*	No	No	Some species present	North America	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease.	(Sindermann 1976)
Aspergillus awamori (black gill infection)	Various penaeid species including: *Penaeus indicus* *Penaeus monodon* *Penaeus vannamei*	No	No	No	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease.	(Karthikeyan, Gnanamoorthy & Gopalakrishnan 2014; Karthikeyan, Selvakumar & Gopalakrishnan 2015)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
Atkinsiella dubia	Various marine crustaceans	No	No	Yes	Japan United States of America	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Lester & Paynter 1989)
Batrachochytrium dendrobatidis	Infects over 350 amphibian species	Yes	Yes	Yes	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia. It is included on the Australian *List of reportable diseases of aquatic animals* and subject to control. However, there is little evidence to indicate that *Batrachochytrium dendrobatidis* would be associated with imported prawns. *Batrachochytrium dendrobatidis* associated with prawns has been reported. However, it is questionable whether crustacean species are susceptible to *Batrachochytrium dendrobatidis*. Given its importance and impact on amphibian species worldwide, it has been included in the table for completeness. The department will continue to monitor the situation with respect to susceptibility of prawn species to *Batrachochytrium dendrobatidis*.	(AHC 2018; 2013; Paulraj et al. 2016; Pessier et al. 2017; Rowley, Alford & Skerratt 2006; Rowley et al. 2007)
***Cladosporium* species**	Various aquatic animals including octopus and penaeid and caridean species such as: *Macrobrachium amazonicum*	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens & Hall-Mendelin 1990)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
***Enterocytozoon hepatopenaei* (EHP)** (hepatopancreatic microsporidiosis (HPM) and enterosporidiosis)	Various penaeid species including: *Penaeus japonicus* *Penaeus merguiensis* *Penaeus monodon* *Penaeus stylirostris* *Penaeus vannamei*	No	Yes	No	Asia Ecuador Honduras	No: disease agent not identified in Prawn IRA 2009	**Yes:** EHP is associated with significant disease in Asia and is included on the Australian *List of reportable diseases of aquatic animals,* and the *List of diseases in the Asia-Pacific.* This pathogenic agent complies with the criteria described in the WOAH *Aquatic animal health code Article 2.1.2 Hazard Identification* and will be retained for risk assessment.	(AHC 2018; Aranguren, Han & Tang 2017; Hudson, Hudson & Pyecroft 2001; Ma et al. 2019; NACA 2016; Salachan et al. 2017; Tang et al. 2017; Thitamadee et al. 2016; Tourtip et al. 2009)
***Fusarium* species** Includes *F. solani*. (burn spot disease, black spot disease, black gill disease and fusariosis)	Various aquatic animals including finfish, decapod crustaceans, carp, and sea turtles	No	No	Yes	France Japan Malaysia Mexico Philippines United Kingdom United States of America	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995; Pillai & Bonami 2012; Yao et al. 2021)
Gilbertella persicaria	*Penaeus monodon*	No	No	No	Asia North and South America	No: disease agent not identified in Prawn IRA 2009	**No.:** not considered to cause significant disease. It has only been reported once in association with farmed *P. monodon* in India, and linked to unfavourable conditions including polluted water, high density and overfeeding.	(Karthikeyan & Gopalakrishnan 2014)
Inodosporus microsporidian (Including *Inodosporus fujiokai n. sp.* and *Inodosporus octospora*)	*Palaemon paucidens* *Palaemon serratus* Also reported in Bima trout and rainbow trout	No	Uncertain	No	Japan United Kingdom	No: disease agent not identified in Prawn IRA 2009	**No:** there is little evidence to indicate that *Inodosporus* species would be associated with imported prawns. *I. fujiokai* has only been reported in *P. paucidens* from Lake Biwa, Japan.	(Stentiford et al. 2018; Yanagida et al. 2022)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							I. octospora has been reported in *P. serratus* from UK waters.	
***Lagenidium* species** (larval mycosis)	Various aquatic animals including marine crustaceans, penaeid and caridean species such as: *Macrobrachium rosenbergii* *Penaeus monodon*	No	No	Yes	Bangladesh Central America India Philippines South America United States of America	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication. *Lagenidium* species have been associated with significant disease in overseas countries when general farming practices (such as washing eggs or nauplii in clean seawater) are not followed.	(Humphrey 1995; Owens & Hall-Mendelin 1990; Pillai & Bonami 2012)
***Leptolegnia* species** Includes *L. marina.* Also known as *Leptolegniella marina and Salilagenidium marinum*)	Crustaceans including: *Penaeus monodon*	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995)
***Leptomitus* species**	Various aquatic animals including freshwater and marine crustaceans	No	No	No	China France India United Kingdom United States of America	No	**No:** not considered to cause significant disease.	(Lightner 1993)
Microsporidian species Includes *Ameson* species, *Agmasoma* species, *Perezia* species, *Pleistophora* species, *Thelohania* species and *Tuzetia* species. Excludes *Enterocytozoon hepatopenaei*.	Various decapod crustaceans	No	No	Yes	Widely distributed	No	**No:** not considered to cause significant disease.	(Glazebrook, Owens & Campbell 1986; Owens & Glazebrook 1988)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
(cotton shrimp disease, milk shrimp disease)								
***Ovipleistophora arlo n. sp.* microsporidian**	*Palaemon serratus*	No	Uncertain	No	United Kingdom	No: disease agent not identified in Prawn IRA 2009	**No:** there is little evidence to indicate that *Ovipleistophora aelo* would be associated with imported prawns. *O. arlo* has only been reported in P. serratus from UK waters.	(Stentiford et al. 2018)
***Pythium* species**	Various aquatic animals including freshwater and marine crustaceans	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995)
***Saprolegnia* species**	Various aquatic animals including freshwater and marine crustaceans	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995)
***Sirolpidium* species** Also known as *Haliphthoros* species. (larval mycosis, brown spot disease)	Various mollusc and crustacean species including penaeids	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Lightner 1985; Meyers 1990)
Yeast	–	–	–	–	–	–	–	–
Yeast species Includes *Candida albicans, Candida sake, Debaryomyces hansenii, Metschnikowia artemia, Metschnikowia bicuspidate, Metschnikowia kamienskiiM*	Various aquatic animal species including salmon and crustaceans, including: *Macrobrachium rosenbergii* *Eriocheir sinensis*	No	No	Yes	Widely distributed	No	**No:** not considered to cause significant disease.	(Baoa et al. 2020; Chen et al. 2003; Chen et al. 2007; Lu, Tang & Chen 1998; Pillai & Bonami 2012)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
etschnikowia pulcherrima and *Saccharomyces cerevisiae*.	*Portunus trituberculatus*							
Protozoa	–	–	–	–	–	–	–	–
Apostome ciliates Includes *Ascophrys* species, *Gymnodinoides* species, *Synophrya* species and *Hyalophysa* species.	Penaeid species. (Other benthic decapods serve as hosts)	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Frischer et al. 2017; Landers et al. 2020; Lutz 2019; Owens et al. 1988; Paynter 1989)
Bodo-like flagellates *Chrysidella* species	Octopods and decapods	No	No	No	Widely distributed	No	**No:** not considered to cause significant disease.	(Humphrey 1995)
Gregarines Includes *Cephalolobus* species, *Nematopsis* species and *Paraophioidina* species.	Various mollusc and penaeid species including: *Penaeus esculentus* *Penaeus merguiensis*	No	No	Yes	Widely distributed	No	**No:** not considered to cause significant disease.	(Jones 1998; Owens 1986)
Haplosporidan species Excludes *Bonamia* species. (hepatopancreatic haplosporidiosis)	Various penaeid species including: *Penaeus duorarum* *Penaeus esculentus* *Penaeus monodon* *Penaeus vannamei*	No	No	Some *Haplosporidan* species are present	Canada Cuba Indonesia Mexico Nicaragua Philippines Thailand United States of America	No	**No:** some *Haplosporidan* species are present in Australia. *Haplosporidan* species associated with Hepatopancreatic haplosporidiosis are not considered to be *Bonamia* species.	(DykovÁ, Lom & Fajer 1988; Jones 1998; Lightner 1996b; Nunan et al. 2007; Thitamadee et al. 2016; Utari et al. 2012)
Haplosporidian-like parasite (red gill disease)	*Macrobrachium nipponense*	No	Unknown	Some *Haplosporidan* species are present	China	No: disease agent not identified	**No**: Red gill disease is thought to be associated with haplosporidian-like parasites found in the gills of *M. nipponense.*	(Ding et al. 2019)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
						in Prawn IRA 2009	Haplosporidian-like parasite associated with red gill disease has not been considered to be *Bonamia* spp and it is considered a potentially new haplosporidian pathogen due to its unique spore ornamentation. The department will continue to monitor the situation with respect to red gill disease.	
***Hematodinium* species**	Various crab species, some penaeid and caridean species including: *Exopalaemon carinicauda* *Penaeus monodon*	No	No	Some members of the genus present	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Gornik, Cranenburgh & Waller 2013; Hudson & Shields 1994; Wang et al. 2017b; Xu et al. 2010)
***Hematodinium*-like species** Also known as spot prawn parasite (SPP).	Various caridean species including: *Pandalus borealis* *Pandalus platyceros*	No	No	No	Canada United States of America	No	**No:** there is little evidence to indicate that spot prawn parasite would be associated with imported prawns.	(Bower & Meyer 2002)
***Leptomonas* species**	Decapods including: Penaeid species	No	No	No	India United States of America	No	**No:** not considered to cause significant disease.	(Humphrey 1995; Lightner 1996b)
***Paramoeba*-like species**	*Penaeus vannamei* Various crustacean, echinoderm and finfish species	No	No	Yes	Widely distributed	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Han 2019)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
***Parauronema* species**	Various marine molluscs and crustaceans including: *Penaeus aztecus*	No	No	No	United States of America	No	**No:** not considered to cause significant disease.	(Bower, McGladdery & Price 1994; Couch 1978)
Peritrichous and loricate ciliates Includes *Cothurnia* species, *Epistylis* species, *Lagenophrys* species, *Rhabdostyla* species, *Stylohedra* species, *Vorticella* species and *Zoothamnium* species.	Marine and freshwater crustaceans including: *Callinectes sapidus* *Cherax rotundus setosus* *Jasus edwardsii* *Macrobrachium* species *Penaeid* species	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995; Paynter 1989)
Scuticociliates Includes *Metanophrys sinensis.*	*Macrobrachium rosenbergii*	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Piazzon, Leiro, & Lamas, 2014) cited in (Sahoo et al. 2018)
Suctorian ciliates Includes *Acineta* species, *Ephalota* species and *Terebrospira* species.	Marine and freshwater crustacea including: *Palaemon* species *Palaemonetes* species *Penaeus* species	No	No	No	Widely distributed	No	**No:** not considered to cause significant disease.	(Humphrey 1995)
***Thalassomyces* species**	Decapods	No	No	No	Widely distributed	No	**No:** not considered to cause significant disease.	(Humphrey 1995)
Metazoa	–	–	–	–	–	–	–	–
***Anisarthus* species**	Caridean species including: *Athanas* species	No	No	No	Japan	No	**No:** not considered to cause significant disease.	(Nakashima 1995)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
***Anisorbione* species**	Various penaeid species	No	No	Yes	Philippines	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Paynter 1989)
***Ascarophis* species**	*Homarus americanus* *Penaeus merguiensis*	No	No	Yes	Barents Sea (Northern Scandinavia Russian Federation) United States of America	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens 1987)
***Austogathona* species**	*Macrobrachium* species	No	No	Yes	Not reported	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Brock 1983, cited in Jennings and Newman 1996) (Paynter 1986)
***Bopyrella* species** Includes *Bopyrinella albida.*	Various caridean and mollusc species	No	No	Yes	Japan	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995)
Bulbocephalus inglissi	*Penaeus merguiensis*	No	No	Yes	Indo-West Pacific West Africa	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens 1987)
Cabirops orbionei	Various penaeid species	No	No	Yes	Indo-West Pacific Red Sea South Africa	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens 1993a)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
***Diceratocephala* species**	Decapod crustaceans	No	No	Yes	New Guinea	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Edgerton et al. 2002)
***Epipenaeon* species**	Various penaeid species, including: *Penaeus semisulcatus*	No	No	Yes	India Indo-West Pacific Israel Persian Gulf Red Sea South Africa Suez Canal Türkiye	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens & Glazebrook 1985)
***Eutetrarhynchus* species**	Various penaeid species	No	No	Yes	India Tunisia	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens 1987)
***Hemiarthus* species**	Various caridean species including: *Pandalus* species *Spirontocaris* species	No	No	No	Greenland Japan United States of America	No	**No:** not considered to cause significant disease.	(Nakashima 1995)
Ionella maculate	*Callianassa* species	No	No	No	New Caledonia Tropical Indo-Pacific	No	**No:** not considered to cause significant disease.	(Markham 1994)
Kronborgia caridicola	Various crustaceans including caridean species and ampeliscid amphipod	No	No	No	Greenland	No	**No:** not considered to cause significant disease.	(Meyers 1990)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
***Metaphrixus* species**	Various caridean species including: *Palaemonella* species	No	No	Yes	New Caledonia Singapore United States of America	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995)
***Microphallus* species**	Various marine crustaceans including penaeid species	No	No	No	United States of America	No	**No:** not considered to cause significant disease.	(Meyers 1990; Owens 1987)
***Nectonema* species**	Various decapod crustaceans including caridean and brachyuran species	No	No	No	Canada New Zealand Norway United States of America	No	**No:** not considered to cause significant disease.	(Meyers 1990)
Opecoeloides fimbriatis	Various marine crustaceans including penaeid species	No	No	No	United States of America	No	**No:** not considered to cause significant disease.	(Owens 1987)
***Opecoeloides* species**	Various aquatic animals including finfish species, and penaeid and caridean species such as: *Macrobrachium australiensis* *Penaeus vannamei*	No	No	Yes	Mexican Pacific United States of America	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Cribb 1987; Owens 1987)
Orbione halipori	Various penaeid species	No	No	Yes	Indo-West Pacific	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
Parachristianella dimegacantha	Various penaeid species	No	No	No	Gulf of Mexico	No	**No:** not considered to cause significant disease.	(Owens 1987)
Parachristianella monomegacantha	Various penaeid species and bivalve mollusc	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens 1987)
***Parapenaeon* species**	Various penaeid species	No	No	Yes	Indo-West Pacific Pakistan	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens & Glazebrook 1985)
Parapenaeonella lamellate	Various penaeid species	No	No	Yes	China Hong Kong India	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens & Glazebrook 1985)
***Polypocephalus* species**	Various penaeid species including: *Penaeus merguiensis*	No	No	Yes	Widely distributed	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995; Owens 1987; Owens & Glazebrook 1985)
***Probopyrus* species**	Various caridean species including: *Palaemonoidea* species	No	No	Yes	India Malaysia United States of America	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Glazebrook, Owens & Campbell 1986)
Prochristianella penaei	Various penaeid species Stingray: *Dasyatis sabina*	No	No	Yes	Gulf of Mexico	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic*	(Owens 1987)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							animals and is not subject to control or eradication.	
Pseudophyllodistomum johnstoni	Freshwater finfish species and caridean species including: *Macrobrachium* species	No	No	Yes	Japan	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995)
Rhadinorhynchids	*Penaeus merguiensis* Various marine finfish Cephalopodo: *Ommastrephes bartrami*	No	No	Yes	Fiji Japan Northwest Pacific Ocean Peru Vietnam	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens 1987)
***Sacculina* species**	Various brachyuran species	No	No	Yes	Ireland Japan Mediterranean Coast Sweden Taiwan Türkiye United Kingdom	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Humphrey 1995)
Sylon hippolytes **(prawn syloniasis)**	Caridean species including: *Pandalina brevirostris* *Pandalus platyceros* *Spirontocaris lilljeborgii*	No	No	No	Canada Faroe Islands Japan Norway United States of America	No	**No:** not considered to cause significant disease.	(Meyers 1990; Nagler et al. 2017)
Temnocephala carpentariae	*Macrobrachium* species *Pomacea canaliculata*	No	No	Yes	Argentina	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic*	(Damborenea 1996; Humphrey 1995)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
							animals and is not subject to control or eradication.	
***Thynnascaris* species** Also known as *Contracaecum* species and *Hysterothylacium*.	Various penaeid and caridean species	No	No	Yes	Barents Sea (Northern Scandinavia Russian Federation) Canada El Salvador Mexican Pacific United States of America	No	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens 1987)
Undetermined aetiology	–	–	–	–	–	–	–	–
Abdominal segment deformity disease (ASDD) Likely associated with a retro virus-like agent.	*Penaeus indicus* *Penaeus vannamei*	No	No	No	India Indonesia Malaysia Philippines Thailand	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease. Aetiology is unknown. The department will continue to monitor the situation with respect to ASDD.	(Janakiram et al. 2018; NACA 2016; Sakaew et al. 2008; Sakaew et al. 2013; Santander-Avancena et al. 2017)
Aggregated transformed microvilli (ATM)	*Penaeus monodon* *Penaeus vannamei*	No	No	No	Thailand	No: disease agent not identified in Prawn IRA 2009	**No:** ATM is not a disease but rather a pathological process caused by transformation and sloughing of microvilli of hepatopancreatic tubule epithelial cells that leads to the accumulation of ATM in the tubule lumens. Massive production of ATM has been linked to gross signs of white faeces syndrome (refer White faeces syndrome).	(Sriurairatana et al. 2014; Thitamadee et al. 2016)
Appendage deformity syndrome	*Macrobrachium rosenbergii*	No	No	No	India	No: disease agent not identified	**No:** not considered to cause significant disease and whilst the aetiology is unknown, it is likely	(Kumar, Rao & Rao 2004; Pillai & Bonami

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
						in Prawn IRA 2009	associated with nutritional deficiency. The department will continue to monitor the situation with respect to appendage deformity syndrome.	2012; Pillai et al. 2005)
Blue body syndrome	*Penaeus vannamei*	No	No	No	China	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease and whilst the aetiology is unknown, it is likely associated with midgut microbiota disruptions.	(Liang et al. 2020)
Branchiostegal blister disease (BBD) Also known as balloon disease.	*Macrobrachium rosenbergii*	No	No	No	India	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease. Aetiology is unknown. The department will continue to monitor the situation with respect to BBD.	(Pillai & Bonami 2012; Pillai et al. 2005)
Cotton shrimp-like disease	*Penaeus vannamei*	No	No	Some *Rickettsiales* and *Tenacibaculum* are present in Australia	China	No: disease agent not identified in Prawn IRA 2009	**No:** aetiology is unknown but is speculated to be associated with *Rickettsiales* and *Tenacibaculum*. The department will continue to monitor the situation with respect to cotton shrimp-like disease.	(Zhou et al. 2019)
Empty stomach disease	*Penaeus vannamei*	No	No	No	China	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease. Aetiology is unknown. The department will continue to monitor the situation with respect to empty stomach disease.	(Li et al. 2016)
Exuvia entrapment disease (EED) Also known as moult death syndrome (MDS) and	*Macrobrachium rosenbergii*	No	No	No	Asia	No: disease agent not identified	**No:** not considered to cause significant disease and whilst the aetiology is unknown, it is likely	(FAO 2020; Pillai & Bonami 2012;

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
metamorphosis moult mortality syndrome						in Prawn IRA 2009	associated with nutritional deficiency. The department will continue to monitor the situation with respect to EED/MDS.	Soundarapandian & Varadharajan 2013)
Idiopathic muscle necrosis	Various penaeid and caridean species including: *Macrobrachium rosenbergii* *Palaemon serratus*	No	No	No	Thailand United Kingdom United States of America	No: disease agent not identified in Prawn IRA 2009	**No:** not considered to cause significant disease. Aetiology is unknown. The department will continue to monitor the situation with respect to idiopathic muscle necrosis.	(Nash, Chinabut & Limsuwan 1987; Pillai & Bonami 2012)
Loose shell syndrome (LSS)	*Penaeus monodon* *Penaeus vannamei*	No	No	No	Bangladesh India	No: disease agent not identified in Prawn IRA 2009	**No:** Aetiology is unknown. The department will continue to monitor the situation with respect to LSS.	(AftabUddin et al. 2017; Alavandi et al. 2008; Han et al. 2019c)
Mid-cycle disease (MCD) *Enterobacter aerogenes* and *Vibrio alginolyticus* have been associated with the disease.	*Macrobrachium rosenbergii*	No	No	Yes	Brazil Hawaii Malaysia Mauritius Philippines Thailand	No: disease agent not identified in Prawn IRA 2009	**No:** present in Australia, is not included on the Australian *List of reportable diseases of aquatic animals* and is not subject to control or eradication.	(Owens & Hall-Mendelin 1990; Pillai & Bonami 2012)
Running mortality syndrome	*Penaeus vannamei*	No	No	No	India	No: disease agent not identified in Prawn IRA 2009	**No:** whilst previously associated with white faeces disease, later reports suggest this is an environmental/husbandry practices issue and not associated with a disease agent.	(Alavandi et al. 2019)
Secret death disease	*Penaeus vannamei*	No	No	No	China	No: disease agent not identified	**No:** aetiology is unknown.	(Li et al. 2016)

Pathogenic agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No)	Considered a hazard in 2022? (Yes/No: reason)	Reference(s)
						in Prawn IRA 2009	The department will continue to monitor the situation with respect to secret death disease.	
White faeces syndrome Also known as white faeces disease and septic hepatopancreatic necrosis (SHPN).	*Penaeus monodon* *Penaeus vannamei*	No	No	Some *Vibrio* species present	China India Indonesia Latin America Malaysia Thailand Vietnam	No: disease agent not identified in Prawn IRA 2009	**No**: aetiology is unknown but it is thought to be associated with infection with EHP (refer *Enterocytozoan hepatopenaei*) and a wide range of *Vibrio* species. Some of the *Vibrio* species are present in Australia and are not subject to official control programs. It has also been attributed to full intestinal ecosystem alterations, rather than a single pathogen. The department will continue to monitor the situation with respect to white faeces syndrome.	(Alfiansah et al. 2020; Aranguren et al. 2019; Aranguren et al. 2021b; Aranguren et al. 2020c; Huang et al. 2020b; Limsuwan 2010; Mastan 2015; Sathish Kumar et al. 2022; Sriurairatana et al. 2014; Tang et al. 2016; Towers 2016; Tran et al. 2017; Wang et al. 2020a)

3.2 Pathogenic agents retained for risk review

The pathogenic agents identified as hazards and retained for risk review were:

- "*Candidatus* Hepatobacter penaei"
- covert mortality nodavirus
- decapod iridescent virus 1
- *Enterocytozoon hepatopenaei*
- infectious myonecrosis virus
- Laem-Singh virus
- Taura syndrome virus
- *Vibrio parahaemolyticus* strains containing Pir toxins
- white spot syndrome virus
- yellow head virus genotypes 1 and 8.

4 Risk assessment methodology and summary of general considerations

This chapter provides the risk assessment methodology and a summary of the general considerations and key assumptions used by the department when undertaking this risk review. An expanded discussion of the general considerations is detailed in Appendix D.

4.1 Evaluating and reporting likelihoods

Likelihood estimations made in this assessment were based on information available in the scientific literature, unpublished data, as well as the expert judgement of the department and other experts.

This risk review used a qualitative approach. The likelihood of entry, exposure or establishment and spread occurring was evaluated and reported using qualitative likelihood descriptors as described in Table 7.

Table 7 Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition
High	The event would be very likely to occur
Moderate	The event would occur with an even probability
Low	The event would be unlikely to occur
Very low	The event would be very unlikely to occur
Extremely low	The event would be extremely unlikely to occur
Negligible	The event would almost certainly not occur

Estimating the likelihoods associated with entry, exposure and establishment and spread involved examining the various factors that influence those likelihoods. For example, the ability of the hazard to remain infectious in frozen product is a key factor in determining the likelihood of the hazard entering Australia in a shipment of prawns.

Entry and exposure likelihood estimations consider the likelihood of the event occurring over a one-year period. This is considered a sufficient period to enable evaluation of seasonal effects, but not so long as to incorporate effects that may be associated with significant changes in disease factors, host factors or factors associated with trade. Entry and exposure assessments for each hazard considered the expected annual volume of trade in the commodity. The previous year's trade was the basis for the expected annual volume of trade. There were no changes in import conditions in the 2021 calendar year to consider when using this data to estimate the expected volume of trade. Table 4 shows the volume of prawns and prawn products exported to Australia during the 2021 calendar year (1 January 2021 to 31 December 2021).

4.2 Entry assessment

The entry assessment determines the annual likelihood of entry into Australia of each hazard. In this risk review, consideration is given to the single-entry scenario which is the importation (from all countries) into Australia of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for

human consumption (imported prawns). It is considered that this commodity type represents the highest biosecurity risk.

Three key factors are relevant in determining the likelihood of viable and infective hazards being present in prawns imported into Australia. These key factors are:

- biological characteristics of the hazards in harvested prawns
- likelihood of detection and removal of infected prawns by post-harvest inspection and grading, or on-arrival inspection as part of the imported food inspection scheme (IFIS)
- ability of the hazard to remain infectious through processing, transport and storage.

4.2.1 Key considerations of the entry assessment

The absence of a pathogenic agent from a region is an important consideration in an entry assessment. The scope of this risk review includes importation of prawns from all countries. Therefore, the entry assessment assumes that the hazards are present in all source countries. Country, compartment or zone freedom from hazards are considered as a biosecurity measure during risk evaluation (see section 5.1.1 Sourcing from free populations).

The assumptions and key considerations made for the entry assessments were:

- The amount of a pathogenic agent in prawns exported to Australia depends on many factors, including the species of prawn, the pathogenic agent, its tissue tropism in the prawn, the production system, and the stability of the pathogenic agent during and post-processing.
- Post-harvest inspection and grading procedures typically focus on human health concerns and the appearance of the commodity to the consumer. They are not likely to remove prawns which are sub-clinically infected or with mild gross signs and therefore cannot be relied upon to reduce entry likelihood.
- IFIS cannot be relied upon to reduce the likelihood of entry of imported prawns infected with hazards. This is because IFIS focuses on food safety issues, not biosecurity issues, such as the presence of *Vibrio cholerae* and uses a compliance history for determining the referral rates for these tests. Once a compliance history is established there can be low inspection rates for cooked and uncooked prawns under this system.
- Washing prawns during processing will likely reduce the number of organisms located on the shell; however, it may also facilitate contamination within and between processing runs. The significance of such transfer and the effectiveness of washing will vary with the pathogenic agent.
- Imported prawns are stored and transported frozen. Once frozen, the amount of any pathogenic agent present is relatively stable; however, the impact of freezing on the viability of the pathogenic agents will be hazard specific.

4.2.2 Estimation of likelihood of entry

The entry assessments considered the factors in section 4.2.1 and estimated the annual likelihood of each hazard entering Australia in imported prawns. The entry assessment used the qualitative likelihood descriptors described in Table 7.

The outcome of the entry assessment was the annual likelihood of entry (LR) into Australia of the hazard.

4.3 Exposure assessment

The exposure assessment determines the likelihood of direct exposure of a susceptible population (exposure group) in Australia to each hazard via potentially infected imported prawns (or associated wastes). The exposure assessment does not consider exposures such as farmed crustaceans exposed to infected wild crustaceans. This exposure is considered when determining the likelihood of establishment and spread during the consequence assessment (see section 4.5 Consequence assessment). All estimates of the likelihood of exposure assume the hazard is present in the imported prawns at the time of arrival in Australia.

The factors considered when estimating the likelihood of an exposure group encountering a hazard, for each major exposure pathway (from entry into Australia, through storage, transport, end-use and any associated waste disposal), included the:

- likelihood of imported prawns (or associated wastes) entering the general environment of the exposure groups
- amount of infectious hazard in imported prawns (or associated wastes) at point of exposure
- contact between susceptible host animals and imported prawns (or associated wastes).

4.3.1 Identification of exposure groups

The three exposure groups considered in this risk review are:

- farmed crustaceans
- hatchery crustaceans (encompassing crustacean hatchery broodstock and postlarvae as well as crustaceans in research facilities and public aquaria)
- wild crustaceans.

There are reports of CMNV infecting some finfish species, including *Carassius auratus* (Wang et al. 2019) and *Danio rerio* (Wang et al. 2021a) which are present in Australia, although neither are native species. Consequently, the definition of the exposure groups in the CMNV risk assessment takes this into account. Further information is provided in chapter 7 Covert mortality nodavirus risk review.

4.3.2 Identification of exposure pathways

Prawns imported for human consumption may be sold to consumers, become waste or be diverted to other uses. Exposure pathways that are direct and that have a high probability of completion contribute substantially to the total likelihood of exposure occurring.

This risk review identified two major pathways as substantially contributing to the total risk:

1) Use of imported prawns as bait or berley for recreational fishing.
2) Use of imported prawns as feed for crustacean broodstock and for crustaceans in research facilities and public aquaria.

The minor exposure pathways (refer Appendix E) are considered unlikely to add appreciably to the overall risk and any biosecurity measures that are necessary to mitigate the major exposure pathways would also likely be sufficient to manage the minor pathways. Therefore, the minor exposure pathways are not considered further in this risk review. Table 8 provides an overview of the major and minor exposure pathways.

Table 8 Summary of the major and minor exposure pathways for imported prawns

Significance	Pathway		Key points
Major	1.	Use of imported prawns as bait or berley for recreational fishing	• Significant and direct pathway for introduction of hazards into the aquatic environment. • Nationally, prawns are the most widely used bait type (Moore et al. 2022). • Recreational fishers use prawns, intended for human consumption, as fishing bait or berley (Moore et al. 2022). • Quality is the main driver for purchasing prawns from a seafood retailer for use as bait or berley (Moore et al. 2022). • Whole uncooked prawns are the preferred form of prawns for use as bait (Moore et al. 2022). These are assumed to be Australian origin because of the current import conditions. • Prawns which have had the head and shell removed, and cooked prawns, are both used as bait by recreational fishers, with a minor amount of usage of value-added products being found (Moore et al. 2022). • Most likely pathway for wild crustaceans to be exposed to hazards.
	2.	Use of imported prawns as feed for crustacean broodstock and for crustaceans in farms, research facilities and public aquaria	• Direct risk pathway for introduction of hazards into farms, hatcheries, research facilities and public aquaria. • Least likely pathway for prawns in farms or hatcheries to be exposed to hazards. • Most likely pathway for crustaceans in research and public aquaria to be exposed to hazards.
Minor	1.	Disposal of solids and liquid waste from commercial processing of imported prawns	• Uncooked imported prawns are not permitted to be further processed unless within an approved arrangement (AA). • AA sites utilised for this purpose are required to manage all associated biosecurity risks, including waste disposal. • No processing of imported prawns currently occurs in Australia on a regular basis.
	2.	Human consumption	• Viable hazard in imported prawns would be reduced or eliminated in the human gastrointestinal tract. • Remaining viable hazard would be further reduced by dilution with other wastes and the physico-chemical environment of domestic sewerage systems.
	3.	Use of imported prawns in the manufacture of pelletised feed for crustacean aquaculture	• Viable hazards in imported prawns used in the manufacture of pelletised feed for crustacean aquaculture would be inactivated by heat treatments associated with feed manufacture.
	4.	Prawn waste disposed at controlled landfill sites	• Viable hazards in imported prawn waste disposed at controlled landfill sites are expected to be inactivated by the conditions at landfill sites.
	5.	Food scraps discarded directly into the aquatic environment	• Most hazards in remains of imported prawns following a meal are expected to be at least partially inactivated by the cooking process. • Remaining viable hazards are likely to be consumed by non-susceptible species eating scraps.
	6.	Prawn wastes disposed through municipal sewerage systems	• Viable hazards in imported prawn waste disposed of in sewerage systems will be significantly reduced, or eliminated, by the treatments and dilution of effluent.

Significance	Pathway	Key points
	7. Discharge of domestic processing effluent into freshwater	• Viable hazards in discharge of processing effluent are expected to be significantly reduced by the effluent treatments required by Australian states and territories to protect public health and the environment.
	8. Other minor pathways	• Includes disposal of imported prawn packaging material, diversion of prawns for use as agricultural fertiliser or as an ingredient in non-aquatic animal feed and chitin production.

Significance: Major exposure pathway that is direct and has a high probability of completion, contributes substantially to the total likelihood of exposure occurring. **Minor** exposure pathway that has a much lower probability of completion because inactivation of the hazard occurs before potential exposure or because it involves only indirect exposure of the aquatic environment.

4.3.3 Key considerations of the exposure assessment

The general assumptions and key considerations made for the exposure assessments were:

- Crustaceans in farms or hatcheries are generally stocked at relatively high densities and are unlikely to have competition from non-susceptible species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced directly into farms and hatcheries would be consumed by susceptible species.
- The probability of wild crustaceans encountering imported prawns (or associated wastes) depends on several factors, including the volume of product released into the natural environment, the dispersal and dilution of that material, the presence and concentration of susceptible crustaceans in the area, and the proportion of material that might be consumed by other non-susceptible species in the vicinity.
- Wild susceptible crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley.
- Wild crustaceans are considered moderately likely to encounter prawn material introduced into their environment. For hazards with a wide host range such as white spot syndrome virus (WSSV), the likelihood of wild susceptible crustaceans encountering that hazard is greater in comparison to those hazards with a smaller host range, such as infectious myonecrosis virus (IMNV).
- There are aquaculture and wild-caught species present in Australia that are susceptible to the hazards outlined in this risk review.
- The amount of infectious hazard present in imported prawns at the point of exposure will depend on numerous factors, including the infectious dose and pathogenic agent stability.
- For this review, of the three exposure groups and the two major exposure pathways, the most likely exposure scenario is:
  - That wild susceptible species are exposed to imported prawns used as bait or berley by recreational fishers.

Use of imported prawns as bait or berley for recreational fishing

The assumptions and key considerations of relevance to the use of imported prawns as bait or berley for the recreational fishing exposure pathway made for the exposure assessments were:

- The regular introduction of prawn material into the aquatic environment through use as bait or berley represents the most likely pathway by which wild crustaceans would be exposed to potentially infected imported prawns.
- This risk review assumes that uncooked imported prawns intended for human consumption will be used as bait or berley by recreational fishers (including uncooked prawns which have had the head and shell removed), unless their availability or form renders them substantially less desirable (for example, processed products).
  - This assumption is supported by the data from the *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* report (2019–20 Bait and berley survey report) (Moore et al. 2022).
- It is important to note that there was a total of 23,747 participants in the 2019–20 Bait and berley survey -online component. From the 23,747 participants, 17,596 responded to questions regarding their fishing participation. A subset of 11,849 participants indicated they fished within the last 12 months. A further subset of 5,514 recreational fishers indicated they had used any type of bait in the previous 12 months. Those who used prawns or shrimp (3,396) were asked if they had purchased the prawns from a seafood retailer or a bait supplier. Those recreational fishers who reported using prawns purchased from a seafood retailer (822) were asked about the prawn (product) type, source (Australian origin or imported) and reasons for purchasing prawns from a seafood retailer rather than a bait supplier (Moore et al. 2022).
  - The department considers the fishers who fished in the past 12 months (11,849) to be the population of interest in considering likelihood and usage proportions of prawns intended for human consumption.
- It was found that nationally, 42% of survey respondents who used some type of bait in the past 12 months, used prawns. Of those, 20.3% indicated that they had purchased the prawns from seafood retailers. The survey found that of the respondents who purchased prawns as bait from a seafood retailer in the 12 months prior to the survey:
  - 82% reported using whole, uncooked prawns (58% reporting using <1 kg: and 24% reporting using >1 kg).
  - 40% reported using uncooked but shelled prawns (34% reporting using < 1 kg; 6% reporting using > 1 kg).
  - 41% reported using cooked but not shelled prawns (27% reporting using < 1 kg; 14% reporting using > 1 kg).
  - 32% reported using cooked and shelled prawns as bait (22% reporting using < 1 kg; 10% reporting using > 1 kg).
  - 24% reported using processed prawns[1] (for example, skewers, marinated, breaded or battered, part of a dumpling, spring roll or other product, or butterflied) (18% reporting using <1 kg; and 6% reporting using > 1 kg) (Moore et al. 2022).
- Of prawn product types used as bait, the origin (Australian or imported) is unknown.
  - It is assumed that whole, uncooked prawns are of Australian origin given the import of this product type is restricted to whole, uncooked prawns from New Caledonia which are imported as a high value, low volume commodity primarily for the restaurant trade.
  - Other prawn types may be of Australian or imported origin.

[1] Skewered, butterflied and marinated prawns are considered uncooked prawns by the department, despite there being a level of processing. Therefore, the usage of breaded, battered and crumbed and dumpling-type products as bait is likely less than what is reported in these usage patterns.

- The survey asked respondents that used prawns as bait sourced from a seafood retailer whether they had used Australian-origin or imported prawns.
  - 78% reported either often using Australian prawns or sometimes using Australian prawns (45.8% and 32.7%, respectively).
  - 46.8% reported they did not use imported prawns.
  - 9.4% reported they often used imported prawns and 24.1% indicated they sometimes used imported prawns (Moore et al. 2022).
- Unintentional introduction may occur on farms where appropriate inlet filtration systems are not in place and imported prawns are used as bait in and around farm inlet and outlet channels. Whilst there have been improvements in entry-level biosecurity on farms in some regions, this still represents a direct pathway for farmed crustaceans.

Use of imported prawns as feed for crustacean broodstock and for crustaceans in farms, research facilities and public aquaria

The assumptions and key considerations of relevance to the use of imported prawns as feed for crustacean broodstock and for crustaceans in farms, research facilities and public aquaria exposure pathway made for the exposure assessments were:

- Feeding of broodstock kept on farms with whole uncooked prawns does not occur in Australia (Australian Prawn Farmers Association 2021; Department of Agriculture and Fisheries 2021).
  - Farmed crustaceans are considered the least likely exposure group to encounter imported prawns.
- Crustaceans kept in hatcheries, research institutions and public aquaria were considered overall unlikely to be deliberately or inadvertently exposed to imported prawns used as bait or berley. This is due to the more stringent biosecurity and physical containment implemented in these facilities.
  - The use of imported prawns as feed to condition broodstock in crustacean hatcheries also does not occur (Australian Prawn Farmers Association 2021; Department of Agriculture and Fisheries 2021).
  - The use of imported prawns as feed for crustaceans in research institutions and public aquaria is unmonitored and unregulated, and represents a potential exposure pathway.

4.3.4 Estimation of partial likelihood of exposure

The likelihood that each exposure group would be exposed to a hazard through contact with imported prawns (or associated wastes) is the partial likelihood of exposure (PLE).

The outcome of the exposure assessment was an estimation of the PLE for each exposure group (described using the nomenclature in Table 7).

4.4 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure (PALEE) is the likelihood that there would be one or more host exposure events over a period of one year. This likelihood was determined for each of the three exposure groups.

The PALEE for each exposure group was calculated by combining the likelihood of entry (LR) (see section 4.2.2 Estimation of entry assessment) and the corresponding partial likelihood of exposure

(PLE) (see section 4.3.4 Estimation of partial likelihood of exposure) using the matrix for combining descriptive likelihoods (see Figure 3).

Figure 3 Matrix for determining the partial annual likelihood of entry and exposure

Likelihood of entry (LR)	Partial likelihood of exposure (PLE)					
	High	Moderate	Low	Very low	Extremely low	Negligible
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate	Moderate	Low	Low	Very low	Extremely low	Negligible
Low	Low	Low	Very low	Very low	Extremely low	Negligible
Very low	Very low	Very low	Very low	Extremely low	Extremely low	Negligible
Extremely low	Extremely low	Extremely low	Extremely low	Extremely low	Negligible	Negligible
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

4.5 Consequence assessment

The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring (OIE 2021d).

For this risk review, the steps taken to assess the 'likely consequences' associated with each hazard were:

- Identifying a likely outbreak scenario that may occur from host exposure to the hazard.
- Estimating the likelihood of that outbreak scenario occurring to obtain a 'partial likelihood of establishment and spread' for the outbreak scenario.
- Determining the level and magnitude of adverse impacts (economic, environmental and social) resulting from the outbreak scenario.
- Combining the 'partial likelihood of establishment and spread' with the corresponding estimation of impacts to obtain the 'likely consequences' for each exposure group.

4.5.1 Identification of the outbreak scenario

Several possible outbreak scenarios may follow exposure of a susceptible population to a hazard. These scenarios represent a continuum ranging from no spread to establishment and spread of the disease to its natural geographic limits. For this risk review, one outbreak scenario was assessed because it has the most potential to occur with significant consequences:

> The hazard establishes in the directly exposed population and spreads to wild and farmed populations, is not eradicated, becomes endemic in Australia and eventually spreads to its natural geographical limits.

4.5.2 Partial likelihood of establishment and spread associated with the outbreak scenario

When estimating the 'partial likelihood of establishment and spread' (PLES) of each hazard several factors were considered, including the:

- likelihood that susceptible species would be exposed to an infectious dose
- mechanisms of spread and transmission pathways
- presence and density of susceptible species
- likelihood that infected animals would be removed by non-susceptible species.

4.5.3 Key considerations for the estimation of partial likelihood of establishment and spread

The assumptions and key considerations made when estimating the PLES for each hazard were:

- The likelihood that a hazard will establish, and spread is affected by how easily the hazard can be transmitted from an index case to other susceptible species. This is influenced by the dose of the hazard necessary to cause infection and the likelihood that susceptible animals would be exposed to that dose.
- The environmental conditions at the time of infection, the density of susceptible animals and the health and immunological status of the recipient host animal are also important factors.
- Transmission from an index case to other susceptible species may occur through ingestion of infected animals or exposure to free hazard (including in faeces) in the water column. The amount of each hazard present in the environment (through for example, shedding by infected animals and dilution of effluent water before release), especially in the case of waterborne transmission, will also affect the likelihood of establishment and spread.
- The dispersal of pathogenic agents can occur via several pathways such as natural migration and movement of infected broodstock or larvae between farms and hatcheries, or water release from infected facilities.
- For most pathogenic agents of prawns, infection usually occurs due to the introduction of a live infected host into a naive (and susceptible) population. Either from shedding of the pathogenic agent into the water or via ingestion of infected host tissues. Transmission from broodstock to progeny has been reported for some pathogenic agents of prawns.
- Disease establishment and spread is more likely in the case of farmed and hatchery crustacean populations, than in wild crustacean populations. This is because of the high density of susceptible animals who would be exposed to an index case, the environmental conditions associated with intensive husbandry practices, and the absence of predators to remove diseased animals in farmed and hatchery crustacean populations.
- In the wild, consumption of diseased prawns by non-susceptible animals may reduce the likelihood of establishment and spread. Factors such as an environment conducive to increased protection from non-susceptible predators would increase the likelihood of establishment and spread in a wild population.
- Indirect exposure routes are more likely for farmed crustaceans than hatchery or wild crustaceans. For example, ineffective or absent biosecurity measures on farms such as crab netting or appropriate inlet filters would increase the likelihood that infected wild crustaceans may enter the farm and cause infection, resulting in establishment and spread of the hazard. Other opportunities for transmission from the wild to the farm include movement of water into the pond that contains infectious hazards due to shedding of pathogenic agents into the water from infected wild crustaceans.
- Spread of a hazard to crustaceans in hatcheries (and research or public aquaria) from wild or farmed crustaceans would generally be least likely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.

- Water transmission for the hazards is considered very effective. If a hazard were to establish in a farm, doses of the hazard sufficient to cause disease would be present in the water column and could spread to other farms and wild crustacean populations through release of untreated effluent water into shared waterways.

4.5.4 Estimation of partial likelihood of establishment and spread

The likelihood of the outbreak scenario occurring for each exposure group is the PLES. The PLES for each exposure were estimated using the qualitative likelihood descriptors in Table 7.

4.5.5 Adverse (economic, environmental and social) impacts

The potential adverse impacts of establishment and spread may be direct or indirect. They were evaluated against seven (two direct and five indirect) impact criteria.

Impacts may occur over an extended period and consideration of them is not limited to what might occur for one year but covers the period the impacts are discernible. The direct and indirect impacts described collectively cover the economic, environmental and social impacts of an outbreak—the so-called 'triple bottom line'. In assessing direct and indirect impacts, impacts were not considered more than once and the frame of reference was the impact of each hazard on the Australian community, rather than on the directly affected parties.

Direct impacts

Direct impacts are those on:

- the life or health (including production effects) of domestic or feral animals
- the environment, including life and health of native wild animals and direct effects on the non-living environment.

Indirect impacts

Indirect impacts are those on:

- new or modified eradication, control, surveillance or monitoring and compensation strategies or programs
- domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries
- international trade, including loss of markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand
- indirect effects on the natural environment, including biodiversity, endangered species, and the integrity of ecosystems
- indirect effects on communities, including reduced tourism, reduced rural and regional economic viability, loss of social amenity, and any 'side effects' of control measures.

Key considerations for the direct and indirect impacts are summarised in Table 9.

Table 9 Summary of the key considerations for each of the direct and indirect impact criterion

Effects	Criteria	Key considerations
Direct	Animal health (production losses in aquaculture and commercial fisheries)	• The biological effect of disease is typically evaluated in terms of morbidity and mortality. • Diseases that reduce the efficiency of production without causing large increases in mortality are more likely to have a significant impact in farmed prawns than wild-caught prawns. • Population fluctuations can be linked quite closely to factors other than 'baseline' mortality, such as fishing pressure, using data collected in studies of population density, age/size structure and catch rates. However, only major epidemics involving significant mortalities or grossly visible clinical signs are likely to be detected in wild populations. • There is evidence that farmed prawn populations may rapidly develop tolerance or resistance to pathogenic agents that initially cause serious disease. The consequences of establishment of an exotic disease in Australian prawn aquaculture is assessed in relation to characteristics of the local industry. • The burden of impacts of an outbreak of an exotic prawn disease in Australia would be felt significantly more in the state(s) or territory(s) where the outbreak occurred, even when the impact is determined as being at a national level. • The Australian prawn farming industry produces over 8,700 tonnes (2020–21) of product annually with a value estimated at over AU$159 million (2020–21) (Tuynman & Dylewski 2022). It provides over 300 full time equivalent jobs, with projections for 1200 direct and 2500 indirect regional jobs within the next 5 years (Presentation by APFA of 8 February 2017 – internal document). • Farming of *P. monodon* accounts for the majority of prawn farming production in Australia. • Aquaculture contributes 34.9% of total Australian prawn production volume (Tuynman & Dylewski 2022). • Wild-caught prawns were worth AU$236 million (volume 16,258 tonnes) in 2020–21 (Tuynman & Dylewski 2022). Other commercially important crustacean species for fishery and aquaculture production include freshwater crayfish, marine lobsters and crabs. In 2020–21, rock lobster contributed 29.1% (AU$403 million) to the wild-caught gross value of fishery production (Tuynman & Dylewski 2022). • This risk review assumes that farmed and wild prawns (including native species) in Australia would be at least as susceptible to infection as prawns of the same species, reported as susceptible under similar conditions in other countries.
	The environment (native animals/plants, and non-living environment)	• The establishment of a new disease could affect the survival of native species not farmed or otherwise commercially exploited. • To determine the likely effect of hazards on Australian native species, the department considered whether the hazards could infect a wide range of species, genera or families.
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	• Australia has a highly developed animal health system that can thoroughly investigate disease problems. • Contingency planning for emergency aquatic animal diseases is well advanced at the national level. The department leads and coordinates the national management of aquatic animal health in Australia. • The costs associated with controlling and monitoring a crustacean disease outbreak would be substantial for the Australian, state and territory Governments and to the prawn farming and fishery industries (refer section 1.5.2 Economic impact of the white spot disease outbreak for further details).

Effects	Criteria	Key considerations
		• A conservative approach was taken in this risk review, considering the high cost and time associated with attempts to eradicate new aquatic animal diseases and the challenges of success.
	Economic (domestic trade effects and impact on other associated industries)	• A disease outbreak may also have additional economic effects due to the loss of domestic markets, market oversupply and resulting reduction of prices received for product. Associated industries including processors, retailers, and the bait industry (for example, prawns and bloodworms) may also suffer significant production losses. • Indirect impacts would also likely affect farms that are free of infection and would be most felt in those parts of Australia where crustacean farming (particularly prawn farming) makes a significant contribution to the overall local economy. • Public perception can significantly affect the markets for products intended for human consumption. For example, infection with WSSV can cause visible lesions on the carapace of infected prawns (Takahashi et al. 1994). Affected product would be unacceptable to the consumer for reasons of quality and aesthetic appeal. • Domestic trade and movement restrictions may apply to wild susceptible species fished from areas impacted by an outbreak. Commercial wild catch industries also include yabbies, bugs, bloodworms, beachworms and mud crabs along with lobsters, crayfish and other crab species. • It is not easy to quantify 'production' in the context of recreational fisheries. Although spending by recreational fishers is likely to provide economic and social benefits to rural and regional areas.
	Economic (international trade effects)	• In 2020–21, Australia exported more than 4,000 tonnes of prawns (from both aquaculture and wild fisheries sectors) valued at AU$73 million (Tuynman & Dylewski 2022). • Several countries have implemented strong import requirements or prohibited the importation of live, fresh and frozen prawns to prevent disease incursions. • The establishment of WSSV, acute hepatopancreatic necrosis disease (AHPND), yellow head virus genotype 1 (YHV1), Taura syndrome virus (TSV), decapod iridescent virus 1 (DIV1) or IMNV in Australia might have an adverse impact on export markets for Australian prawns, both live and non-viable. • If an exotic disease were to become established, Australia could use zoning to maintain access to international markets for live crustaceans including prawns and, if required, non-viable product, noting that importing countries may not necessarily accept zoning arrangements.
	Environment (biodiversity, endangered species and the integrity of ecosystems)	• The potential loss of biodiversity if a hazard were to be introduced, establish and spread, would be of concern to the Australian community. • A conservative approach was taken by the department when considering the susceptibility of native species, particularly those that are endangered or threatened, to infection with the hazards. • In drawing conclusions on the likely impact of exotic disease on the environment, the department considered overseas data on the species of prawns and other crustaceans that are susceptible, the effect of infection and the influence of the physical environment on the outcome of infection.

Effects	Criteria	Key considerations
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	• In the event of a disease outbreak, communities where prawn farming is a significant employer and/or plays a major role in the local community, are expected to experience the most significant social impacts. • Loss of social amenity by recreational fishers because of the implementation of a movement regulated or restricted area could occur. • Loss of culturally significant species will also have social impacts if a disease outbreak were to affect important species for indigenous cultural fishing.

4.5.6 Determining impacts

Estimating the 'overall impact' associated with the outbreak scenario involved a two-step process where first, a qualitative descriptor of the impact of the hazard was assigned to each of the direct and indirect criteria in terms of the *level of impact* and the *magnitude of impact*. The second step involved combining the impacts for each of the seven criteria to obtain an 'overall impact' estimation.

Step 1: Assessing direct and indirect impacts

Each direct and indirect impact was estimated over four geographic levels, defined as:

- Local—an aggregate of households or enterprises (a rural community, a town or a local government area).
- District or region—a geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory, such as 'Far North Queensland').
- State or territory—a geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia).
- National—Australia wide (Australian mainland states and territories and Tasmania).

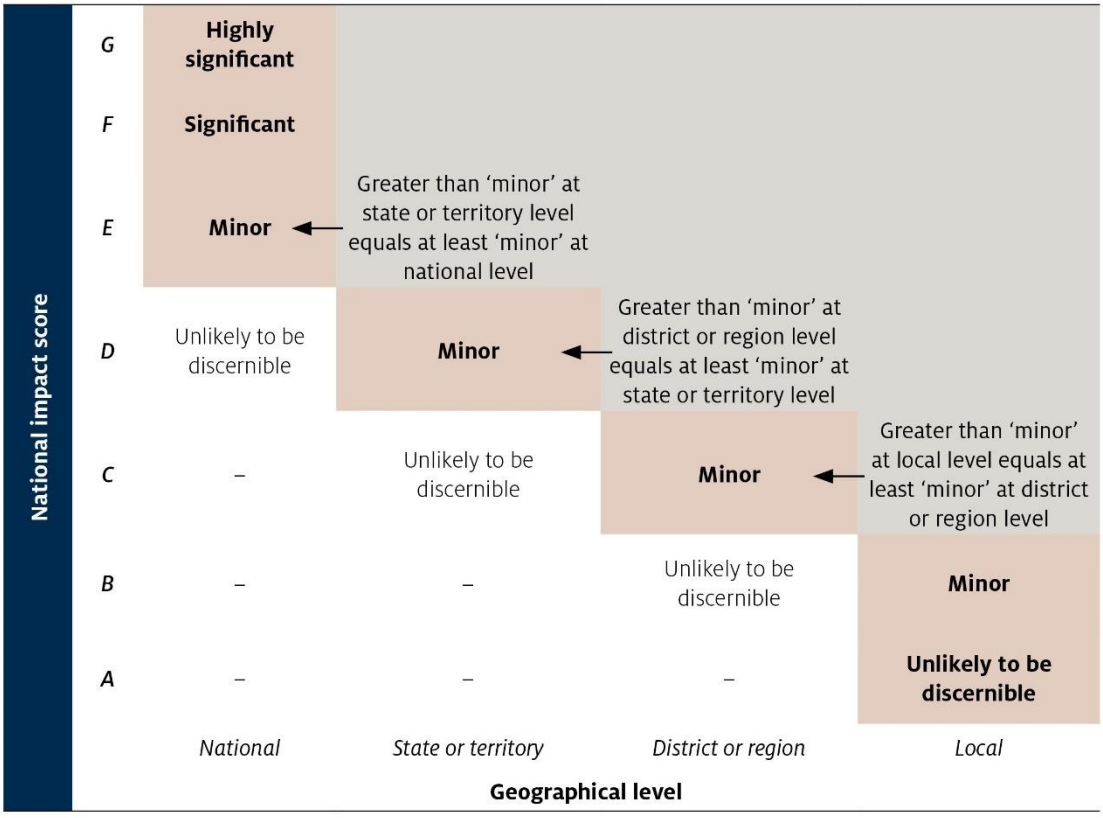
At each level, the magnitude of impact was described using four categories, defined as:

- Unlikely to be discernible—impact is not usually distinguishable from normal day-to-day variation in the criterion.
- Minor significance—impact is recognisable, but minor and reversible.
- Significant—impact is serious and substantive, but reversible and unlikely to disturb either economic viability or the intrinsic value of the criterion.
- Highly significant—impact is extremely serious and irreversible and likely to disturb either economic viability or the intrinsic value of the criterion.

Each individual direct or indirect impact was given an impact score (A–G) using the schema outlined in Figure 4. This was done by determining which of the shaded cells with bold font in Figure 4 corresponded to the level and magnitude of the particular impact. Additionally:

- At each geographic level below national, an impact more serious than 'minor' is considered at least minor at the level above. For example, a 'significant' impact at the state or territory level is considered equivalent to at least a 'minor' impact at national level.
- If the impact of a disease at a given level is in more than one state or territory, district or region or local area, it is considered to represent at least the same magnitude of impact at the next highest geographic level. For example, a 'minor' impact in multiple state or territories represents a 'minor' impact at national level.
- The geographic distribution of an impact does not determine the impact. For example, an outbreak could occur on one farm, but the impact could potentially still be considered at a state or national level.

Figure 4 Assessment of direct and indirect impacts on a national scale



Step 2: Combining direct and indirect impacts

The impact scores (A-G) for each direct and indirect criterion were combined to determine the 'overall impact' using the rules in Table 10. These rules are mutually exclusive and are assessed in numerical order until one applies. For example, if the first rule does not apply, the second rule is considered, and so on.

Table 10 Rules for combining direct and indirect impacts

Rule	Impact scores for each direct and indirect criteria	Overall impact
1	Any criterion has an impact of 'G'; or more than one criterion has an impact of 'F'; or a single criterion has an impact of 'F' and each remaining criterion an 'E'.	Extreme
2	A single criterion has an impact of 'F'; or all criteria have an impact of 'E'.	High
3	One or more criteria have an impact of 'E'; or all criteria have an impact of 'D'.	Moderate
4	One or more criteria have an impact of 'D'; or all criteria have an impact of 'C'.	Low
5	One or more criteria have an impact of 'C'; or all criteria have an impact of 'B'.	Very Low
6	One or more but not all criteria have an impact of 'B', and all remaining criteria have an impact of 'A'.	Negligible

4.5.7 Determination of likely consequences for outbreak scenario

'Likely consequences' for the outbreak scenario were determined by using the matrix in Figure 5 to combine the 'overall impact' (see Step 2: Combining direct and indirect impacts in section 4.5.6) with the 'likelihood of establishment and spread' (see section 4.5.4 Estimation of partial likelihood of establishment and spread).

When interpreting the matrix, note the vertical axis refers to *'likelihood of establishment and spread (PLES)'* and the horizontal axis refers to '*consequences of establishment and spread (impact score)'*. Accordingly, a '*low*' PLES combined with '*high*' impact, is not the same as a '*high*' PLES combined with '*low*' impact. This is because the matrix is not symmetrical.

Figure 5 Matrix for determining the 'likely consequences' for the outbreak scenario

Likelihood of establishment and spread (PLES)	Consequences of establishment and spread (impact score)					
	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
Very low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Extremely low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low

4.6 Estimation of overall annual risk

'Risk estimation' is the integration of 'likelihood of entry and exposure' and 'likely consequences' to derive the overall risk associated with entry, establishment and spread of a hazard.

Risk estimation was undertaken in two stages:

- determining the partial annual risk (of entry, exposure, establishment and spread) for each of the three exposure groups
- combining the three partial annual risks to give an estimate of 'overall annual risk'.

4.6.1 Determination of partial annual risk

The partial annual risk (PAR) is the annual risk associated with each exposure group.

The PAR is determined by combining the PALEE (see section 4.4 Determination of the partial annual likelihood of entry and exposure) with the estimate of 'likely consequences' (see section 4.5.7 Determination of likely consequences for outbreak scenario) using the risk estimation matrix (Figure 6).

When interpreting the matrix, note the vertical axis refers to '*likelihood of entry and exposure (PALEE)*' and the horizontal axis refers to *'consequences of entry and exposure ('likely consequences')*'. Accordingly, a '*low*' PALEE combined with '*high*' likely consequence, is not the same as a '*high*' PALEE combined with '*low*' likely consequence. This is because the matrix is not symmetrical.

Figure 6 Matrix for determining the partial annual risk

Likelihood of entry and exposure (PALEE)	Consequences of entry and exposure ('likely consequences')					
	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
Very low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Extremely low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low

4.6.2 Estimation of overall annual risk

The overall annual risk is obtained by combining the PAR (see section 4.6.1 Determination of partial annual risk) for each of the exposure groups using the six rules outlined in Table 11.

These rules are mutually exclusive and are addressed in the order that they appear in the list. For example, if the first rule does not apply, the second rule is considered, and so on.

Table 11 Rules for combining partial annual risks

Rule	Partial annual risks of the exposure groups	Overall annual risk rating
1	any one partial annual risk is extreme; or more than one partial annual risk is high; or any one partial annual risk high and each remaining partial annual risk is moderate.	Extreme
2	a single partial annual risk is high and the remaining partial annual risks are not unanimously moderate; or all partial annual risks are moderate.	High
3	one or more partial annual risks are moderate; or all partial annual risks are low.	Moderate
4	one or more partial annual risks are considered low; or all partial annual risks are very low.	Low
5	one or more partial annual risks are very low.	Very Low
6	all partial annual risks are negligible.	Negligible

The result of this process was an estimate of the overall annual risk of introducing a hazard through importation of non-viable, whole, farm-sourced, frozen, uncooked prawns intended for human consumption. This is the final output of the unrestricted risk assessment.

4.7 Risk management

Australia has traditionally maintained a very conservative attitude to biosecurity risk. Given this, an overall annual risk that is either 'very low' or 'negligible', is sufficiently conservative to achieve Australia's ALOP. This provides a benchmark for evaluating risk and determining whether biosecurity measures are required.

4.7.1 Evaluating risk management options

The process for using a benchmark for evaluating risk is:

- For each hazard, the level of risk associated with the unrestricted importation of prawns was estimated using the process described in this chapter.
- The unrestricted risk was then evaluated to determine where it fell in relation to Australia's ALOP.
- If the unrestricted risk was 'negligible' or 'very low', then it was considered acceptable and further biosecurity measures were not required for that hazard.
- If the unrestricted risk was 'low', 'moderate', 'high' or 'extreme', then biosecurity measure(s) were identified (see chapter 5 Options for biosecurity management of imported prawns) and the risk was recalculated (referred to as 'restricted risk') with the biosecurity measure(s) applied.
- Where the subsequently restricted risk was 'very low' or 'negligible', that biosecurity measure(s) was considered acceptable for that hazard.

4.7.2 Cost/benefit analysis of risk management options

As a member of the World Trade Organization, and as a partner in free trade agreements, Australia has obligations to allow trade where the science says it is safe to do so. When considering risk management options, the department considers whether the biosecurity measure achieves Australia's ALOP and if it is operationally feasible. Any biosecurity measure which the department considers practical and effective at managing risk must be offered as an option for importers, exporters and therefore consumers. This means there will often be multiple import conditions for the one commodity. For example, whilst cooking may achieve ALOP for imported prawns, if other biosecurity measures also achieve ALOP, it is appropriate to offer all these options to importers. It becomes a decision for importers, and ultimately the consumer, as to whether the cost for them to import a product is financially and commercially viable. This is not a decision for the department to make. Whilst cooking is the only risk management measure used at the domestic level to manage the risk of WSSV in prawns sourced from the Queensland movement regulated area, internal movement controls put in place by the states and territories are at the discretion of those jurisdictions. In addition, the practicalities of implementing risk management measures are an important consideration when implementing internal movement controls.

5 Options for biosecurity management of imported prawns

Biosecurity measures considered in this risk review are aimed at reducing the likelihood that the importation of prawns for human consumption from any country would lead to the entry, exposure, establishment and spread of hazards in Australia. There are two means by which this may be achieved:

- Reducing the likelihood of hazards entering Australia in imported prawns by imposing conditions that would reduce the likelihood of entry.
- Reducing the likelihood that susceptible host animals in Australia would be exposed to the hazard in contaminated imported prawns or associated waste by imposing conditions that would reduce one or more of the partial likelihoods of exposure.

The least trade restrictive biosecurity measures that could be applied to achieve Australia's appropriate level of protection (ALOP) were evaluated in the Prawn IRA 2009 and these are reviewed here, along with the current import conditions and consideration of new biosecurity measures.

These biosecurity measures were selected from a range of pre-export and on-arrival measures considered practicable and form the basis of the biosecurity measures that are recommended to apply to the importation of prawns for human consumption (see chapter 16 Proposed biosecurity measures for imported prawns). Alternative biosecurity measures that are demonstrated, to the satisfaction of Australian government authorities, to provide equivalent biosecurity would also be considered.

Appendix F provides the risk assessment values of the biosecurity measures found to reduce the overall risk of each hazard to at least *very low*, thereby achieving Australia's ALOP.

5.1 Biosecurity measures considered further

The Prawn IRA 2009 concluded that several biosecurity measures would reduce the overall risk associated with each hazard to achieve Australia's ALOP. A number of those options may still reduce risk to within Australia's ALOP and they are considered further for each hazard during this risk review.

5.1.1 Sourcing from free populations

The Prawn IRA 2009 considered that importation of prawns could be permitted from countries, compartments or zones determined to be free of the hazard(s).

Determination of hazard freedom would need to be to a standard consistent with that recommended by the World Organisation for Animal Health (WOAH, formerly OIE), or equivalent. To be satisfied that a country, compartment or zone is free of a given disease, the department must have formally recognised the competent authority of that country and be satisfied that the competent authority has the capacity for disease control, monitoring and surveillance as appropriate for the disease. It would be necessary for the disease to be subject to compulsory reporting or be the subject of consideration in disease investigation. The WOAH *Aquatic animal health code* (WOAH code) chapter 4.2 *'Zoning and compartmentalisation'* (WOAH 2022m), chapter 1.4, Article 1.4.3 '*Pathways to*

demonstrate freedom from disease' (WOAH 2022c) and the relevant provisions in each disease chapter of the WOAH Code for 'self-declaration of country freedom', should be followed as a guide.

A rigorous assessment of any application for approval of 'sourcing from free populations' would be undertaken to ensure that effective biosecurity measures are implemented and maintained throughout the complete supply chain (from source population to point of export). A detailed submission would need to be provided to the department by the competent authority of the exporting country. Australia would conduct a desk audit followed by an on-ground assessment and verification of the proposed disease-free country, compartment or zone, before the system could be approved. It is still considered that importation from disease-free countries, compartments or zones is expected to reduce the overall risk associated with each hazard to a level that achieves Australia's ALOP.

Importation from disease-free countries, compartments or zones is expected to reduce the entry risk of hazards to a level that achieves Australia's ALOP. However, consideration of this biosecurity measure has not been documented in each risk assessment chapter because it is dependent upon satisfactory assessment of the country's competent authority and its capacity to determine and maintain disease freedom.

5.1.2 Sourcing from wild stocks

In many cases wild-caught prawns would pose a lower biosecurity risk than farmed prawns. The Prawn IRA 2009 considered allowing imported prawns that were sourced from wild-caught populations under alternative conditions. Ultimately, this option was considered generally unfeasible in the Prawn IRA 2009 because it was determined that existing audit procedures in most exporting countries would not facilitate relevant competent authority attestations.

The department now considers that some hazards, including white spot syndrome virus (WSSV), are present at a prevalence and load in wild populations that is of biosecurity concern to the department. Therefore, whilst consideration of alternative biosecurity measures for prawns sourced from wild stocks could occur following a case-by-case assessment, it is likely that wild-caught prawns would need to be assessed as a disease-free population (in-line with the requirements outlined in section 5.1.1 Sourcing from free populations).

5.1.3 Cooking

The Prawn IRA 2009 considered that whole prawns could be permitted import subject to cooking offshore in premises approved by, and under the control of, the competent authority. Alternatively, prawns could be cooked post-arrival, under biosecurity control in Australia.

In the Prawn IRA 2009, a prawn was considered fully cooked when all the protein was coagulated. It was at that point marketability remained, there was assumed to be some pathogen inactivation and the attractiveness of the prawn for other end-uses was significantly reduced. As part of assurance systems, the department closely monitors imports of cooked prawns and prawn products to ensure import conditions continue to manage biosecurity risks appropriately. This can include random inspections to ensure the prawn meat appears fully coagulated.

Cooked prawns that are exported to Australia, regardless of their size, are cooked to achieve a core temperature of at least 65°C. This ensures compliance with conditions required to inactivate bacteria

of concern to food safety, and ensures all the protein in the prawn is fully coagulated so they meet current biosecurity import conditions. For prawns exported to Australia, almost exclusively the cooking process is done through steaming. Processors typically record the core temperature achieved for each batch of cooked prawns (Food & Beverage Importers Association, 2022, pers. comm., 23 August). It is difficult to quantify the cooking of seafood products. Cookbooks may define prawns as being cooked by the surface colour, however this may make the products appear done before being fully cooked internally. Internally the opaqueness of the prawn meat more accurately represents the doneness of the prawns than the external colour does (Brookmire, Mallikarjunan & Jahncke 2010).

The *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* (2019–20 Bait and berley survey) found that cooked prawns were not the preferred form of prawns used as bait or berley by recreational fishers in Australia (Moore et al. 2022). However, the usage of cooked prawns was greater than that assumed in the Prawn IRA 2009. The 2019–20 Bait and berley survey reported that the usage of cooked prawns as bait or berley, was similar to the usage of uncooked, shelled prawns as bait or berley (Moore et al. 2022) and therefore the department now assumes in this risk review that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal.

A study by Winkel (1998) showed that a prawn is fully cooked, based on its appearance, when a core temperature of 70°C is achieved, lower temperatures were not reported. From a food safety perspective, the United States Department of Agriculture's Food Safety and Inspection Services recommends that shellfish attain a minimum core temperature of 62.8°C (USDA Food Safety and Inspection Services 2020). Prawns become opaquer as they approach 63°C. A comparison of various cooking times and temperatures needed to achieve microbial safety and to maintain high quality cooked prawns, found this was optimally achieved at temperatures more than 63°C (63.2–71.0°C), depending upon the size, cooking method and prawn type (Brookmire et al. 2013). Based on this study, a prawn appears fully cooked, internally and externally, after it attains a core temperature of at least 63°C (Brookmire et al. 2013).

The length of time a prawn is cooked (to ensure optimum quality and achieve food safety requirements) depends on several factors such as prawn size, species, cooking method and cooking temperature. In general, the higher the temperature a prawn is cooked at, the less time it needs to be held at that temperature to manage food safety risks and be cooked. From a food safety perspective, the United States, Food and Drug Administration provides guidance on the length of time fish and fishery products need to be held at a specific core temperature for the inactivation of pathogens. For example, prawns should be cooked at 63°C for 17 minutes, 65°C for 9.3 minutes or 72°C for 1 minute to accomplish a six-logarithm reduction in *Listeria monocytogenes* (U.S. Food and Drug Administration 2022). Prawn grades from 11–28 grams (starting temperature of 20°C) placed into boiling water were reported to reach a core temperature of 85°C at 2.40–4.55 mins, respectively (Winkel 1998).

The Prawn IRA 2009 considered that cooking would have an impact on the infectivity of the hazards, although it would be hazard and temperature dependent. That assumption is still considered valid. Cooking is expected to reduce the likelihood of entry, however, the degree to which this would reduce the amount of infectious hazard present in prawns is hazard and temperature specific. For

those hazards sensitive to heat inactivation, cooking will likely significantly reduce the amount of hazard present in prawns. For some more thermostable hazards, cooking is not expected to eliminate the infectious load of the hazard. Scientific knowledge about agent stability has improved for some hazards since the Prawn IRA 2009 was completed, and there is now more robust data to support considerations of core temperature on some agent infectivity. For example, a study completed in 2022 demonstrated that WSSV-infected prawns cooked to a core temperature of 60°C, 70°C, 75°C, 85°C, and 95°C were not capable of causing WSSV-infection in naive prawns (Aquaculture Pathology Laboratory & Department of Agriculture 2022a).

Dependent upon the hazard, it may be necessary to specify a minimum core temperature for cooked prawns to achieve Australia's ALOP. Any specific requirements for a minimum core temperature would need to inactivate or partially inactivate the hazard to reduce the entry likelihood and ensure the prawns appear fully cooked to reduce the exposure likelihood. When recommending a minimum core temperature, the need to provide a tolerance for prawn-to-prawn temperature variation within cooking batches and commercial practicality would also be considered. Therefore, taking these into consideration and to ensure cooked prawns achieve Australia's ALOP for all hazards, this risk review considers prawns to be cooked when they appear fully cooked and have achieved a core temperature of at least 65°C.

In summary, cooking, with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C, would generally be expected to reduce both entry and exposure likelihoods for the hazards. The extent to which this would occur is dependent upon the specific hazard. It is considered that for these reasons, cooking should be considered further for each hazard.

5.1.4 Freezing

Prawns for human consumption are frequently packaged, after sorting, washing and freezing. It is also common for whole prawns to be cooked and then frozen (although this assessment assumes imported prawns are uncooked). Whether cooked or uncooked, rapid freezing is important to maintain quality.

The freezing operations commonly practiced around the world vary considerably according to the type of product. Uncooked whole or head-off prawns may be block or plate-frozen in purpose-designed cartons into which potable water is poured to form a solid block with protective ice. However, Australia does not receive much (if any) of this product type due to the on-arrival sampling methodology currently requiring product to be easily accessible for testing of WSSV and yellow head virus genotype 1 (YHV1). At the other extreme, cooked and peeled cold water prawns tend to be frozen through fluidized bed systems, while many warm water prawns are individually quick frozen (IQF) either on trays in blast freezers or in continuous belt freezers. The Codex Alimentarius *Code of Practice for Fish and Fishery Products* states that storage of frozen prawns should be at or below –18°C (FAO & WHO 2016). Frozen prawns may be held in frozen storage for many months (ADVS 1999).

Freezing will generally reduce the rate of inactivation of microorganisms (ADVS 1999) and is an excellent way to preserve many microbes (Archer 2004). Storage at freezing temperatures kills many food-borne pathogenic protozoa, cestodes and nematodes (Archer 2004). Most viruses are stable at freezing temperatures (Hasson et al. 1995; Lightner et al. 1997b; Lu et al. 1995), but bacteria that are

pathogenic or potentially pathogenic to aquatic species are often inactivated to some degree by freezing (ADVS 1999; Su & Liu 2007).

Once frozen, the amount of most hazards that might be present is relatively stable. However, depending on the agent and the physical conditions, freezing and thawing will reduce the number of viable hazards present.

The Prawn IRA 2009 considered that freezing was a suitable biosecurity measure for "*Candidatus* Hepatobacter penaei" (previously known as necrotising hepatopancreatitis bacterium). Freezing may still be suitable for reducing the risk posed by some of the hazards and is considered in the context that the unrestricted risk is for frozen product.

5.1.5 Value-added products

The Prawn IRA 2009 considered breaded, battered and crumbed (BBC) prawns, dumpling and dim sum-type products and marinated prawns to be 'highly processed prawns'. The Prawn IRA 2009 concluded that 'highly processed prawns' would achieve Australia's ALOP because the exposure risks associated with the use of prawns by recreational fishers as bait or berley or for their use as feed for crustaceans would be reduced. Specific import conditions were then applied to each product type for them to meet the definition of a 'highly processed prawn'.

For the purposes of risk evaluation for each hazard in this risk review, BBC prawns and dumpling and dim sum-type products which contain raw prawns are considered under a single category; 'value-added products'. Evaluation of whether this category manages biosecurity risks will be undertaken for each hazard. Separate import conditions will then apply for each product (see chapter 16 Proposed biosecurity measures for imported prawns) to ensure that biosecurity risks are managed. The 2019–20 Bait and berley survey found that processed prawns (which included breaded or battered prawns or part of a dumpling, spring roll) were the least preferred prawn used as bait or berley. Of the respondents who purchased prawns from a seafood retailer for use as bait in the 12 months prior to the survey, 24% reported using processed prawns, with 18% reporting using less than 1kg in the 12 months prior to the survey, compared to 6% reporting using more than 1kg (Moore et al. 2022).

Breaded, battered and crumbed prawns

The Prawn IRA 2009 considered that uncooked prawns which have had the head and shell removed (the last shell segment and tail fans permitted) and had been coated for human consumption by being breaded, battered or crumbed were 'highly processed prawns'.

Under the import conditions implemented in September 2018 (Biosecurity Advice 2018/15) BBC prawns are those that are coated for human consumption by being breaded, battered or crumbed, the head and shell removed (the last shell segment and tail fans permitted) and have been par-cooked to solidify and adhere the coating to the prawn.

It is considered that the biosecurity risks associated with BBC prawns are managed due to the reduced likelihood of diversion to bait and berley because of the higher value of the product, the form of the product not being suitable for bait or berley use and the preference for (unprocessed) prawn meat as bait. Par-cooking reduces the likelihood of the product being imported for illegal reprocessing into 'uncooked prawn meat' by removal (washing) of the coating. BBC prawns that have

not been par-cooked are not within the scope of this biosecurity measure and are considered an uncooked prawn. BBC prawns would not require virus testing but would be subject to inspection at the border.

BBC prawns are considered under the category of 'value-added products' for the purposes of risk evaluation. Specific import conditions will apply to BBC prawns (see chapter 16 Proposed biosecurity measures for imported prawns) to ensure biosecurity risks are managed.

Dumpling and dim sum-type products which contain uncooked prawns

The Prawn IRA 2009 considered that dumpling, spring roll, samosa, roll, ball or dim sum-type products which contain uncooked prawns (which have had the head and shell removed (the last shell segment and tail fans permitted)) were 'highly processed prawns'.

Under the current import conditions, dumpling and dim sum-type products which contain uncooked prawns (which have had the head and shell removed (the last shell segment and tail fans permitted) and in which the uncooked prawn meat within the product has been processed to the extent that no discernible pieces are salvageable) are permitted import subject to meeting specific requirements. These products do not require virus testing but are subject to inspection at the border.

The biosecurity risks associated with dumpling and dim sum-type products which contain uncooked prawns are managed due to the reduced likelihood of diversion to bait and berley because of the higher value of the product, the form of the product not being suitable for bait use and the preference for (unprocessed) prawn meat as bait.

Dumpling and dim sum-type products which contain uncooked prawns are considered under the category of 'value-added products' for the purposes of risk evaluation. Specific import conditions will apply to dumpling and dim sum-type products which contain uncooked prawns (see chapter 16 Proposed biosecurity measures for imported prawns) to ensure biosecurity risks are managed.

5.1.6 Head and shell removal (last tail segment and tail fans permitted)

The Prawn IRA 2009 determined that removal of the head and shell (last tail segment and tail fans permitted) of uncooked prawns would be expected to reduce the likelihood of entry of some hazards and/or exposure of susceptible populations to the hazards.

The degree to which this biosecurity measure would reduce the amount of hazard present in prawns (and therefore entry risk) is hazard specific. For those hazards present primarily in the head and shell, this measure will likely reduce the amount of hazard present in prawns by at least half. Head and shell removal is not expected to eliminate the hazards, and for those hazards present primarily in the muscle, it would have minimal effect. For many hazards, it is considered that there would still be sufficient hazard present in the tail muscle to cause disease even with the head and shell removed.

The Prawn IRA 2009 also concluded that this measure would reduce the likelihood of exposure in terms of those pathways associated with head/shell disposal or unintended end-use. This was because of the expected higher cost of such a product and the reported preference for head on prawns for use as recreational fishing bait or berley and as feed for broodstock. Current data shows that convenience and quality are the main drivers for recreational fishers purchasing supermarket prawns for use as bait or berley (Biosecurity Queensland 2017; Kantar Public 2017, 2019; Moore et al. 2022). The 2007 survey identified an increase in the use of peeled prawns to bait fishing hooks

(Kewagama Research 2007). This risk review therefore assumes that uncooked imported prawns intended for human consumption will be used as bait or berley by recreational fishers, unless their availability or form renders them substantially unsuitable. That is, it is assumed that the removal of the head and shell will not significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley.

The 2019–20 Bait and berley survey found that of the respondents who purchased prawns from a seafood retailer for use as bait in the 12 months prior to the survey, 40% reported using uncooked but shelled prawns, with 34% reporting using less than 1kg in the 12 months prior to the survey, compared to 6% reporting using more than 1kg. Although the survey shows that uncooked prawns which have had the shell removed are still attractive for use as bait by recreational fishers, they are not used as bait at a rate similar to whole, uncooked prawns which was the assumption in the draft report.

It is considered highly unlikely that imported prawns with the head and shell removed would be used as feed for crustaceans on farms or in the hatchery setting. This is because, notwithstanding the known biosecurity risks of this practice, the primary biological purpose for this behaviour is the use of the head as conditioning feed.

In summary, head and shell removal would generally be expected to reduce both entry and exposure likelihoods for the hazards. The extent to which this would occur is dependent upon the specific hazard (and the exposure group). It is considered that for these reasons, head and shell removal should be considered further for each hazard.

5.1.7 Deveining

Deveining refers to the removal of the intestinal tract of a prawn. Deveined prawns are commonly sold without the head and shell; the tail may or may not be attached.

Whilst it is possible to devein a whole prawn, removal of the gut on its own will not reduce the load of hazards in the rest of the prawn to a level that achieves Australia's ALOP and it is unlikely to reduce the exposure likelihood of whole prawns. Therefore, the department has only considered deveining as a biosecurity measure in combination with head and shell removal (in the circumstance that head and shell removal on its own does not achieve Australia's ALOP).

Deveining of uncooked (head and shell removed) prawns is expected to reduce the likelihood of entry of some hazards. The degree to which this biosecurity measure would reduce the amount of hazard present in prawns is hazard specific. For those hazards present primarily in gut-associated tissues including the midgut and the hindgut, deveining will likely significantly reduce the amount of hazard present in prawns. Deveining of uncooked (head and shell removed) prawns is not expected to completely eliminate the hazards, and for those hazards present primarily in the muscle of the tail, it would have minimal effect. For many hazards it is considered that there would still be sufficient hazard present in the tail muscle to cause disease.

It is not considered that deveined (head and shell removed) prawns are significantly different from non-deveined (head and shell removed) from the perspective of their attractiveness for use as bait or berley, or feed for crustaceans on farms or in the hatchery setting. This is because deveining does not significantly change the cost or physical appearance of the prawns compared to prawns which had

the head and shell removed but not been deveined. Therefore, deveining of uncooked (head and shell removed) prawns is not considered to reduce the likelihood of exposure more than head and shell removal on its own will (see section 5.1.6 Head and shell removal (last tail segment and tail fans permitted)).

The extent to which deveined (head and shell removed) prawns reduce entry risk is dependent upon the specific hazard (and the exposure group). It is considered that for these reasons, head and shell removal plus deveining, should be considered further for each hazard when head and shell removal on its own does not achieve ALOP.

5.1.8 Batch testing for hazards

The Prawn IRA 2009 recommended testing for WSSV and YHV1 in uncooked (head and shell removed) prawns on-arrival in Australia at a laboratory approved by the department as a biosecurity measure. Only those batches (see section 17.1.2 for batch definition) that tested negative for WSSV and YHV1 were eligible for release from biosecurity control (assuming they met all other import requirements).

The department implemented revised import conditions for testing in July 2017 (Biosecurity Advice 2017/12) (Department of Agriculture and Water Resources 2017b). They include that the competent authority is required to certify uncooked (head and shell removed) prawns have been found, post-processing, to be free of WSSV and YHV1. On-arrival in Australia, the prawns are subject to inspection and sampling by biosecurity officers and testing for WSSV and YHV1 by a department approved laboratory. Only those batches, which test negative for WSSV and YHV1, are eligible for release from biosecurity control (assuming they meet all other import requirements). Under current operational practices, the prawns are subject to 100% secure seals intact inspections, however this is an operational means to ensure compliance with import conditions. Equivalent methods to ensure compliance may be considered by the department in the future, including systems such as the compliance-based intervention scheme.

Testing methods should be at least to a standard consistent with the recommendations in the latest version of the WOAH *Manual of diagnostic tests for aquatic animals*, or equivalent. To continue improving the effectiveness of biosecurity measures, the department may specify alternative methods with higher diagnostic sensitivity and/or specificity than the methods recommended by the WOAH, as new methods become available.

In general, the sampling regime should provide 95% confidence of detecting the hazard if it is present at a prevalence of 5% or greater. However, these parameters would be determined for any hazard requiring batch testing (noting these testing parameters are considered appropriate for WSSV and YHV1). In all cases, samples should be representative of the batch of prawns.

The level of protection provided by testing would depend amongst others, on the integrity of the sampling regime (including security of the batches), strict implementation of the sampling procedures (including appropriate random selection of samples), the availability of effective testing methods and the prevalence of the target agent in the batch of prawns. Testing may be applied pre-border (pre-export) or on-arrival (at border). A combination of pre-export and on-arrival testing may also be used to improve the effectiveness of this biosecurity measure.

For the purposes of this risk review, pre-export testing in the country of origin is not considered equivalent to on-arrival testing in Australia, and therefore the magnitude of the reduction in entry likelihood is not equal when considering the biosecurity measures. This is because Australia has not assessed the exporting country's pre-export testing systems.

Systems outside those considered within this risk review would be considered on a case-by-case basis to determine if they are an equivalent biosecurity measure. This might include assessment of pre-export testing programs in the country of export to be used in conjunction with an on-arrival compliance-based inspection program in Australia. The department considers that the application of an on-arrival compliance-based inspection scheme in Australia will be required for any equivalence system implemented (pre-export testing, on-arrival testing or equivalence based programs) unless it is determined by case-by-case assessment that it achieves Australia's ALOP without it. Alternatively, it may be determined that for certain hazards only 100% inspection and testing will achieve Australia's ALOP.

As the effectiveness of testing for managing biosecurity risks may vary for different hazards, this option may be applied in combination with other measures to reduce the overall risk to an acceptable level.

Further details regarding the pre-export and on-arrival testing program for imported prawns, including sample design and testing details, is provided in chapter 17 Testing of imported prawns.

5.1.9 Labelling for human consumption-only

The requirement for labelling of imported prawns "for human consumption only" and "not to be used as bait or feed for aquatic animals" was implemented following the release of the Prawn IRA 2009. It was considered that this measure may reduce the likelihood of exposure by making clear the intended end-use as being for human consumption and prevent diversion at wholesale, including for use as aquatic animal feed, bait or berley. The main benefit of the labelling being that in those cases where the product was no longer considered fit for human consumption and it was downgraded, it was clear it should not be diverted to bait suppliers. Since that time, the department has required that the labelling also be on the primary packaging (that is the retail ready bags), however, this labelling requirement does not necessarily apply at point of sale where loose product is sold (for example, in a fish market or supermarket delicatessen). When purchased as loose product, consumers may not see labelling and when purchased in packaging consumers may not read the labelling. In the Prawn IRA 2009 this option was not considered likely to reduce the overall risk to an acceptable level on its own, although it was a recommended measure.

The conclusion on the requirement for labelling of imported prawns in the Prawn IRA 2009 is still valid and this measure should apply to all imported prawn packaging. The 2019–20 Bait and berley survey found that nationally, 20.3% of respondents indicated that they had purchased bait from seafood retailers in the previous 12 months (Moore et al. 2022). The survey also found that 24.1% of respondents who purchased bait from seafood retailers in the previous 12 months sometimes used imported prawns and 9.4% often used imported prawns, a further 19.7% did not know if they had used imported prawns (Moore et al. 2022).

Any reduction in unintended end-use or deliberate diversion, for example as bait, is beneficial in reducing risk. It is recommended that Australian state and territory governments implement

regulations requiring similar labelling be in place at the point of sale (for example, in situations where loose product is sold). The department intends to define legibility expectations for the labelling of uncooked prawns as part of the department's biosecurity labelling requirements for uncooked prawns.

5.2 Biosecurity measures not considered further

The Prawn IRA 2009 concluded that several biosecurity measures would not reduce the overall risk associated with each hazard to achieve Australia's ALOP. It is considered that a number of those options will still not reduce risk to within Australia's ALOP. Additionally, some biosecurity measures considered suitable to manage risk in the Prawn IRA 2009 no longer reduce risk to a level that meets Australia's ALOP. These options are discussed but are not considered in the risk reviews for each hazard.

5.2.1 Marinated prawns

Prawns that have had the head and shell removed (the last shell segment and tail fans permitted) and were marinated to a minimum standard were considered highly processed prawns under conditions implemented following the release of the Prawn IRA 2009.

Under the import conditions implemented July 2017 (Biosecurity Advice 2017-12), marinated prawns are not considered to meet the definition of highly processed (Department of Agriculture and Water Resources 2017b). This is because the department is now of the view that marination does not substantially change the shape, appearance, form, cost or attractiveness of the prawn for use as bait or berley. This is in part due to the ease with which the products could be returned to an 'unprocessed' form by washing. Therefore, marinated prawns are not considered a highly processed prawn. Marination is not considered further as a biosecurity measure.

5.2.2 Sourcing from non-emergency harvested stock

The Prawn IRA 2009 considered allowing only importation of farmed prawns that have not been emergency harvested (subject to verification by the overseas competent authority) to reduce the amount of hazard present in prawns, and thereby the likelihood of entry and exposure. The extent to which this option would reduce the likelihood of entry and exposure would depend on the specific hazard. The Prawn IRA 2009 did not consider that this measure alone would manage biosecurity risk to an appropriate level. This conclusion is still valid and it is highlighted that many of the hazards are now endemic on farms. Prawns can be infected with hazards at levels that are capable of transmitting diseases without the need for the ponds to have been emergency harvested. Accurate certification of this option is also considered very difficult to be implemented and verified. This option is not considered to reduce overall risk to meet Australia's ALOP and it is not considered further.

5.2.3 Minimum size

Minimum prawn size restrictions were not considered likely to reduce the overall risk to an acceptable level in the Prawn IRA 2009. This option is not considered to reduce overall risk to meet Australia's ALOP and it is not considered further.

5.2.4 Post-harvest inspection to ensure absence of clinical signs of disease

The Prawn IRA 2009 considered that import of prawns could be permitted subject to verification by the overseas competent authority that the prawns showed no signs of clinical disease on post-harvest inspection. This measure should reduce the number of clinically infected prawns and in general terms, reduce the number of prawns containing significant amounts of hazard. However, in the Prawn IRA 2009 it was determined that as many of the hazards can result in sub-clinical infection, the level of risk reduction provided by this option would not be sufficient to manage biosecurity risks on its own. This conclusion is still valid, and this option is not considered on its own. However, application of this measure is best practice, and it will remain a requirement on health certificates.

5.2.5 Irradiation

Irradiation is a physical treatment in which a commodity is exposed to a defined dose of ionising radiation, either from gamma rays or a high-energy electron beam or powerful x-rays (Food Standards Australia & New Zealand 2017). Irradiation can reduce the numbers of pathogenic or spoilage microorganisms in fresh food (Food Standards Australia & New Zealand 2017).

Gamma irradiation is currently accepted by the department as a biosecurity treatment for a range of products of animal origin, for uses other than human consumption. These products include imported bait for aquatic use, pet fish food and aquaculture feed products, containing uncooked prawns and uncooked prawn meat (Department of Agriculture 2014a).

Food Standards Australia and New Zealand (FSANZ) approves the use of irradiation of food intended for human consumption. In Australia, the entry and sale of irradiated meat or aquatic animal products intended for human consumption is not currently permitted (FSANZ 2021). Therefore, irradiation is not considered further as a biosecurity measure. If FSANZ were to approve the use of irradiation in prawns and prawn products intended for human consumption, the use of this biosecurity measure will be reviewed.

6 "*Candidatus* Hepatobacter penaei" risk review

6.1 Background

"*Candidatus* Hepatobacter penaei" ("*Ca.* H. penaei") is the aetiological agent of necrotising hepatopancreatitis (NHP), a disease of penaeid prawns which has caused significant losses in prawn aquaculture in the Western Hemisphere (OIE 2021f).

"*Ca.* H. penaei" is an obligate intracellular bacterium of the order *Rickettsiales* (Nunan et al. 2013). Susceptible host species include various penaeid prawns (OIE 2021f). NHP was first reported in farmed prawns from Texas, United States of America (USA) in 1985 and has since been detected throughout the Americas (Brinez, Aranguren & Salazar 2003; Frelier et al. 1992; Lightner & Redman 1994; Lightner, Redman & Bonami 1992; Loy et al. 1996b; Vazquez-Sauceda et al. 2016). NHP has also been referred to as Texas pond mortality syndrome, Peru NHP and granulomatous hepatopancreatitis (Frelier et al. 1992; Lightner & Redman 1994).

Infection with "*Ca.* H. penaei" is listed as a disease notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c) and is on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Australia has a long history of passive surveillance and a strong system in place to detect incursions, "*Ca.* H. penaei" is considered exotic to Australia.

At the time the *Generic import risk analysis report for prawns and prawn products 2009* (Prawn IRA 2009) was finalised, the aetiological agent of NHP was unclassified and was referred to as NHP-bacterium (NHPB) (Biosecurity Australia 2009). To simplify naming of the hazard in this chapter, "*Ca.* H. penaei" will be used even if the literature being cited referred to NHPB.

6.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of "*Ca*. H. penaei" is warranted.

6.2.1 Agent properties

"*Ca.* H. penaei" is a pleomorphic Gram-negative bacterium classified within the class *Alphaproteobacteria*, order *Rickettsiales* (Nunan et al. 2013). It has been suggested to belong to the *Holosporaceae* family between the *Rickettsiales* (Leyva et al. 2018). Nunan et al. (2013) proposed the classification and provisional naming of "*Ca.* H. penaei" to help eliminate confusion with other pathogenic bacteria that can cause similar pathology of the hepatopancreas in *Penaeus vannamei* (Nunan et al. 2013).

"*Ca.* H. penaei" is an obligate intercellular pathogen that cannot be cultivated in cell-free media (Nunan et al. 2013). It has two morphological variants, a more common rod-shaped rickettsial-like form (0.25 × 0.9 µm) and a motile helical variant with eight flagella located at the basal apex (0.25 × 2–3.5 µm). The basal flagella in the motile helical variant may be an evolutionary adaptation that allows "*Ca.* H. penaei" to pass through the digestive system of the prawn to colonize the

hepatopancreas, and/or to move in the aquatic environment where the prawn hosts live (Nunan et al. 2013).

"*Ca.* H. penaei" can remain infectious in prawns stored at 4°C for up to 2 days (Donald Lightner [The University of Arizona] 2007, pers. comm., 23 March). However, "*Ca.* H. penaei" is considered highly sensitive to freezing and not able to survive normal commercial freezing temperatures (Donald Lightner [The University of Arizona] 2007, pers. comm., 23 March). "*Ca.* H. penaei" requires the use of cryoprotectant (Gracia-Valenzuela et al. 2011) or specially developed fast freezing techniques to maintain infectivity (Luis Fernando Aranguren [The University of Arizona] 2020, pers. comm., 5 February). For example, *per os* exposure and forced-feeding experiments have shown that "*Ca.* H. penaei" frozen in 50% glycerol at –20°C (no ultra-freezing procedures) for up to 14 months can infect juvenile *P. vannamei* (Gracia-Valenzuela et al. 2011). In that case, glycerol was used as a cryoprotectant which allowed the "*Ca.* H. penaei" to retain infectivity (Gracia-Valenzuela et al. 2011). Additional studies were able to reproduce NHP by using prepared homogenates composed of "*Ca.* H. penaei"-infected hepatopancreas and cryoprotectant, which were stored at –20°C (Ávila-Villa et al. 2012a) or – 80°C for up to 6 months (Gollas-Galván et al. 2014). Further *per os* experiments showed that "*Ca.* H. penaei" was transmitted to juvenile *P. vannamei* fed on NHP-affected hepatopancreas, but only when flash frozen at –80°C, and that infectivity of "*Ca.* H. penaei" in tissue was not altered after being flash frozen for up to 80 days (Crabtree et al. 2006). Aranguren et al. (2010) reproduced NHP in two lines of *P. vannamei* by using a "*Ca.* H. penaei"-inoculum flash frozen at −70°C for reverse gavage inoculation (Aranguren, Tang & Lightner 2010). NHP has also been transmitted to prawns after intra-hepatopancreatic injection of a preparation of enriched "*Ca.* H. penaei", obtained by density gradient ultracentrifugation and preserved at –70°C (Frelier, Loy & Kruppenbach 1993).

"*Ca.* H. penaei" has been detected in samples of zooplankton (Mendoza-Cano et al. 2013). As some pathogenic bacteria had been reported to be able to survive and persist in water by their ability to adhere to chitin-containing surfaces (such as those of zooplanktonic organisms), it was suggested that the ability to colonize zooplankton surfaces by "*Ca.* H. penaei" may be an important strategy for its survival in adverse conditions and once released into the extracellular environment (Mendoza-Cano et al. 2013). However, further studies are needed as Mendoza-Cano et al. (2013) did not elucidate whether "*Ca.* H. penaei" was attached to the chitinaceous exoskeleton of zooplankton or was internally distributed in the mid-gut gland (Mendoza-Cano et al. 2013).

6.2.2 Epidemiology

Host range

Species which fulfil the criteria for listing as a species susceptible to infection (N= natural; E= experimental exposure) with "*Ca.* H. penaei" in accordance with chapter 1.5. of the WOAH *Aquatic animal health code* (WOAH Code) (WOAH 2022c) include:

- *Penaeus vannamei* [N, E] (Brinez, Aranguren & Salazar 2003; Crabtree et al. 2006; Frelier et al. 1992; Krol, Hawkins & Overstreet 1991; Lightner & Redman 1994; OIE 2021f; Vincent, Breland & Lotz 2004).

Species for which there is incomplete evidence for listing as susceptible to infection (N= natural; E= experimental exposure) include:

- *Penaeus aztecus* [N] (Aguirre Guzman et al. 2010; Frelier et al. 1994; OIE 2021f)
- *Penaeus duorarum* [N] (Aguirre Guzman et al. 2010; OIE 2021f)
- *Penaeus marginatus* [N] (Brock et al. 1986a; OIE 2021f)
- *Penaeus merguiensis* [N] (Brock et al. 1986a; Lightner & Redman 1985; OIE 2021f)
- *Penaeus monodon* [E] (OIE 2021f; Pantoja & Lightner 2003)
- *Penaeus setiferus* [N, E] (Frelier et al. 1994; OIE 2021f)
- *Penaeus stylirostris* [N] (Lightner & Redman 1994; OIE 2021f).

"*Ca.* H. penaei"-positive PCR results (E = experimental exposure) have been reported in the species:

- *Homarus americanus* [E] (Ávila-Villa et al. 2012b; OIE 2021f).

"*Ca.* H. penaei"-positive PCR results and necrotic spots in the hepatopancreas of the lobster *H. americanus* were found after forced feeding with "*Ca.* H. penaei" (Ávila-Villa et al. 2012b). Based on these results, (Ávila-Villa et al. 2012b) suggested that "*Ca.* H. penaei" is capable of infecting different crustacean species inhabiting diverse latitudes. However, it was noted that the lobsters in the study were maintained under experimental conditions that could have affected the resistance of the lobster to the pathogen and favoured the propagation of "*Ca.* H. penaei" (Ávila-Villa et al. 2012b).

Infection with "*Ca.* H. penaei" has been demonstrated in several stages of *P. vannamei* including larvae, juveniles, adults and broodstock (Aranguren et al. 2006; OIE 2021f).

Geographical distribution

NHP was first reported from prawn farms in Texas, the United States of America in 1985 (Krol, Hawkins & Overstreet 1991) and was subsequently detected throughout the Americas in farmed and wild penaeid prawns. Affected countries include Belize, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru and Venezuela (Aguirre Guzman et al. 2010; Aranguren et al. 2006; Brinez, Aranguren & Salazar 2003; Frelier et al. 1992; Lightner & Redman 1994; Loy et al. 1996b; Vazquez-Sauceda et al. 2016).

NHP was introduced to Eritrea, Africa but later eradicated (Lightner et al. 2012b; Pantoja & Lightner 2003). There have been reports of NHP in Vietnam (AGDAFF–NACA 2007; OIE 2013) and Thailand (Limsuwan & Chuchird 2007).

Prevalence

The average "*Ca.* H. penaei" prevalence in farmed prawns collected from 11 Latin American countries between 2000–2015 was 43% (minimum of 10% and maximum of 80%) (Morales-Covarrubias et al. 2018). Other epidemiological studies in *P. vannamei* and *P. stylirostris* farms in multiple Latin American countries have reported prevalence of 0.43–0.77% in Peru (Cuéllar-Anjel 2013; Ramírez et al. 2020) and 0.6–1.3% in Belize, Brazil, Guatemala, Honduras, Mexico, Nicaragua and Venezuela (Cuéllar-Anjel 2013). In Mexico, during a NHP outbreak in 2002, the prevalence of "*Ca.* H. penaei" from 42 farms in Sinaloa was found to be between 5–42%, and between 14.6–86.2% from 9 farms in Sonora (Ibarra-Gámez, Galavíz-Silva & Molina-Garza 2007). In addition, "*Ca.* H. penaei" prevalence of 40.6% was reported in Mexico from 150 *P. vannamei* sampled from 10 different ponds during an NHP outbreak (Rio Rodríguez et al. 2006).

NHP prevalence in wild prawn populations range from 0–17% in Mexico (Aguirre Guzman et al. 2010; Rio Rodríguez et al. 2006; Vazquez-Sauceda et al. 2016). Grossly normal wild *P. setiferus*, *P. duorarum* and *P. aztecus* were collected randomly from 2 sampling stations in Laguna Madre, Gulf of Mexico. The prevalence of "*Ca.* H. penaei" in the sampled *P. duorarum* was 15% and 5.6%, 17% and 5% in *P. aztecus* and 0% and 0% in *P. setiferus* (Aguirre Guzman et al. 2010). Vazquez-Sauceda et al. (2016) collected wild prawn samples from the San Andres Lagoon, Mexico, and reported a "*Ca.* H. penaei" prevalence of 2.5% (2/80) in the sampled prawns (Vazquez-Sauceda et al. 2016).

Mortalities

Cumulative mortalities due to NHP range from 20–95% in farmed prawns (Loy et al. 1996a). Mortalities of up to 95% have been reported in *P. vannamei* from Texas (Frelier et al. 1992), 70–90% in *P. vannamei* and *P. stylirostris* from Peru (Lightner & Redman 1994) and 20–80% in *P. vannamei* and *P. stylirostris* from Mexico (Rio Rodríguez et al. 2006). NHP-affected ponds of broodstock in Colombia reported mortalities of up to 85%, while non NHP-affected broodstock ponds in the same farm experienced mortalities of 40–50% (Aranguren et al. 2006).

NHP has been detected in wild prawn populations but there are no reports of declines in catch rates or associated mortalities which have been attributed to NHP.

Transmission

Horizontal transmission occurs through ingestion of infected tissues (Crabtree et al. 2006; Vincent, Breland & Lotz 2004; Vincent & Lotz 2005) and ingestion of the agent in water (Frelier et al. 1994; Vincent, Breland & Lotz 2004). "*Ca.* H. penaei" shed into pond water through faeces has been suggested as a source of infection (Brinez, Aranguren & Salazar 2003; Vincent & Lotz 2005). An unpublished study cited by Aranguren et al. (2006) found postlarvae from "*Ca.* H. penaei"-positive females were also "*Ca.* H. penaei"-positive, suggesting transmission from broodstock to progeny occurs. Transmission has also been demonstrated through injection of purified bacteria (Frelier, Loy & Kruppenbach 1993).

No "*Ca.* H. penaei" vectors are currently known in natural infections (OIE 2021f). However, *Navicula* species, *Artemia* species and zooplankton have been proposed. "*Ca.* H. penaei" has been detected in samples of zooplankton from areas with high NHP prevalence by qPCR but it is still unknown whether the "*Ca.* H. penaei" is able to colonize the zooplankton or it is associated with chitin-containing surfaces (Mendoza-Cano et al. 2013). "*Ca.* H. penaei" has been detected by PCR in *Navicula* and *Artemia franciscana* experimentally exposed to "*Ca.* H. penaei". Of those prawns fed on "*Ca.* H. penaei"-positive *Navicula*, 20% were found to be "*Ca.* H. penaei"-positive by PCR (Ávila-Villa et al. 2011).

Mechanism of spread

The introduction of NHP into new areas has been attributed to trade and movement of infected broodstock and postlarvae (Lightner et al. 2012b).

Infected live prawns and whole fresh (not frozen) prawns can effectively transmit "*Ca.* H. penaei" (Frelier et al. 1994), therefore untested live and whole fresh prawns from affected areas may pose a risk of introduction of NHP into new countries or areas. "*Ca.* H. penaei", together with Taura syndrome virus (TSV) was introduced into Eritrea from Mexico via movement of infected *P. vannamei* broodstock (Lightner et al. 2012b; Wertheim et al. 2009). After introduction, NHP became

temporarily established in Eritrea but was later eradicated following depopulation and fallowing (Lightner et al. 2012b). It has been suggested that the nature of "*Ca.* H. penaei" and its requirement for high water temperatures and high salinity (from a prolonged dry season) may be the reason why major prawn producing countries of Asia have remained free of NHP, despite introductions of potentially infected stocks of *P. vannamei* (Lightner & Redman 1994; Lightner et al. 2012b; Morales-Covarrubias et al. 2011; Vincent & Lotz 2005). However other studies have reported that NHP is not influenced by these factors (Vazquez-Sauceda et al. 2016).

Infectious dose

The minimum infectious dose of "*Ca*. H. penaei" required to cause NHP in susceptible species by experimental challenge or natural infection is not known. However, *per os* bioassays demonstrate that NHP can be successfully transmitted to *P. vannamei* fed a 0.05g piece of "*Ca.* H. penaei"-infected hepatopancreas. The amount of "*Ca.* H. penaei" was not determined in the piece of tissue (Vincent, Breland & Lotz 2004; Vincent & Lotz 2005). Successful transmission of NHP was observed in juvenile *P. vannamei* after *per os* exposure by adding 0.04g of "*Ca.* H. penaei"-infected hepatopancreas to each aquarium containing individual prawns and allowing them to feed naturally (Gracia-Valenzuela et al. 2011). Additionally, *P. vannamei* developed NHP and presented mortalities following force feeding with 40µl of an inoculum containing 0.04g of "*Ca.* H. penaei"-infected hepatopancreas (Gracia-Valenzuela et al. 2011). In similar studies, *H. americanus* developed hepatopancreatic necrosis after being forced fed with 1ml inoculum extracted from the hepatopancreas of "*Ca.* H. penaei"-infected prawns and homogenized with glycerol (1:1 v/v) (Ávila-Villa et al. 2012b).

6.2.3 Pathogenesis

Following *per os* ingestion, "*Ca.* H. penaei" moves to its target tissue, the hepatopancreas. The eight basal flagella in the motile helical variant of "*Ca.* H. penaei" may be an evolutionary adaptation that allows the bacteria to pass through the digestive system and to colonize the hepatopancreas, which subsequently causes the pathology seen in NHP (Nunan et al. 2013). Physiological alterations of the hepatopancreas result in mortalities that can reach 90–95% within 30 days of infection (AGDAFF–NACA 2007).

NHP has an acute and a chronic phase. In the acute phase, lesions in affected prawns include necrosis and sloughing of epithelial cells in the hepatopancreas and melanized hepatopancreatic tubules. In the chronic phase, the hepatopancreas lesions are characterized by atrophy of tubules, reduced epithelial cell height, low lipid storage R cells and intratubular oedema (Aranguren & Dhar 2018).

NHP has been reported to cause a reduction in fertility of female broodstock (Aranguren et al. 2006). NHP may impair hepatopancreas function on lipid transfer and storage. The severe hepatopancreas damage might be incompatible with maturation and spawning, as the ovary needs to reach a certain level of lipid reserves to mature and spawn. "*Ca.* H. penaei"-infected female broodstock is also reported to produce nauplii and larva of decreased quality (Aranguren et al. 2006).

Tissue tropism

"*Ca.* H. penaei" targets the hepatopancreas with infection reported in all hepatopancreatic cell types (Lightner et al. 2012b). "*Ca.* H. penaei" is also present in the faeces (Brinez, Aranguren & Salazar 2003; Vincent & Lotz 2005).

Tissue titre

Few studies have attempted to examine the titre of "*Ca.* H. penaei" in infected prawn tissues as the number of DNA copies using qPCR. Prawns with NHP show a massive infection of hepatopancreatic cells by "*Ca.* H. penaei" (Lightner & Redman 1994). NHP was quantified by qPCR in *P. vannamei* (mean weight 5.1g) fed 1 piece of "*Ca.* H. penaei"-infected hepatopancreas (0.05g piece, with an undetermined copy number of NHP). "*Ca.* H. penaei" was detected at 10^3–10^7 copies/mg in hepatopancreas and 10^1–10^5 copies/mg in faeces (Vincent & Lotz 2005). Lethal infections contained 10^6–10^7 copies/mg in hepatopancreas and 10^3–10^6 copies/mg in faeces. The amount of "*Ca.* H. penaei" present in the hepatopancreas was higher than that observed in faeces of the same individual (Vincent & Lotz 2005). In a separate study, quantification of "*Ca.* H. penaei" in hepatopancreas and faeces samples of *P. vannamei* (mean weight 2.8g) by qPCR showed that "*Ca.* H. penaei" copy number ranged from 3.0×10^2–8.8×10^7 copies/µg of DNA in hepatopancreas, and mean copy number of 4.3×10^3–4.2×10^6 copies/µg in faeces (Aranguren, Tang & Lightner 2010).

6.2.4 Diagnosis

Clinical signs

Clinical signs of NHP are nonspecific. NHP is associated with anorexia, lethargy, abdominal muscle atrophy, softened exoskeleton, blackened gills, chromatophore expansion, growth retardation and mortalities (Lightner & Redman 1994; OIE 2021f).

Pathology

The typical histological characteristics of NHP are atrophy, multifocal necrosis and inflammation of the hepatopancreas (Frelier et al. 1994; Lightner & Redman 1994). Infection with "*Ca.* H. penaei" can be diagnosed using histological methods during the acute and chronic phases of infection; the initial phase of infection on the other hand, is difficult to diagnose (OIE 2021f). Acute NHP infection appears as an atrophied hepatopancreas with moderate atrophy of the tubule epithelia, presence of bacterial cells and haemocytic infiltration of the tubules (multifocal encapsulations). Other histological findings during this phase include hypertrophied tubular cells, sloughing of tubule epithelial cells and an irregular content of lipid vacuoles in the hepatopancreatic tubules (OIE 2021f). In transitional NHP infection, an evident atrophy of the hepatopancreas tubule epithelium and haemocytic infiltration are shown. Haemocyte nodules with masses of bacteria in its centre can also be observed. The content of lipid vacuoles in the hepatopancreatic tubules is markedly reduced (OIE 2021f). Chronic phase of NHP infection appears primarily as infiltration and accumulation of haemocytes at the sites of necrosis, low haemocyte nodules, areas with fibrosis, and few melanised and necrotic hepatopancreatic tubules (OIE 2021f).

Testing

Chapter 2.2.3 of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH Manual) (WOAH 2022e) provides details of the methods currently available for targeted surveillance and diagnosis of NHP, in addition to which tests are recommended for targeted surveillance to declare freedom from infection with "*Ca.* H. penaei".

qPCR targeting the 16S rRNA gene is the WOAH recommended method for targeted surveillance to declare freedom from "*Ca.* H. penaei" (OIE 2021f). A new qPCR protocol targeting a region of the "*Ca.* H. penaei" flagella gene (flagella hook protein, flgE) was described by Aranguren and Dhar

(2018) to enhance specificity and avoid non-specific amplifications observed when screening *Artemia* cysts for "*Ca.* H. penaei" with the PCR and qPCR assays recommended in the WOAH Manual.

6.2.5 Treatment

Early detection of NHP is critical for successful treatment, as cannibalism of infected prawns contributes to the spread of infection (Frelier et al. 1994; OIE 2021f). NHP, particularly in the initial phase, can be treated by using antibiotics in medicated feeds (OIE 2021f). "*Ca.* H. penaei" is sensitive to oxytetracycline (Frelier et al. 1994; Lightner & Redman 1994) and florfenicol (Morales-Covarrubias et al. 2012).

6.2.6 Control

Control measures for NHP are primarily aimed at preventing the introduction of "*Ca.* H. penaei" into susceptible populations. The development of specific pathogen free broodstock and screening of wild or pond-reared broodstock by PCR have proven to be effective preventive measures. Other general preventive measures include raking, tilling and removing sediments from the bottom of the ponds, prolonged drying (through exposure to sunlight) of ponds and water distribution canals for several weeks, disinfection of fishing gear and other farm equipment using calcium hypochlorite and extensive liming of ponds (OIE 2021f).

6.2.7 Impact of the disease

Infection with "*Ca.* H. penaei" has caused massive economic losses in the prawn aquaculture sector since 1985 (Krol, Hawkins & Overstreet 1991; Lightner et al. 2012b). In the Americas, NHP has been reported as the most significant disease after white spot syndrome virus (WSSV) and TSV, in terms of production losses and its cost of management in primarily, *P. vannamei* farms (Lightner et al. 2012b). For example, the cumulative losses in Texas between 1985 to 1992 were estimated to be 1,700-7,684 tonnes of stock valued at US$13.83-62.25 million (Shinn et al. 2018b). In Texas, a farm that reported NHP for the first time during the late 1980s was forced to abandon prawn farming activities as a result of the high mortalities (up to 95%) (Frelier et al. 1992). Similarly in Peru, NHP outbreaks in 1993 resulted in the closure of approximately half of the country's active prawn farms (Lightner & Redman 1994) and in loss of sales valued at US$20 million (Shinn et al. 2018b). In Colombia, decreases in nauplii availability was reported to be due to NHP in broodstock (Brinez, Aranguren & Salazar 2003). Also, NHP resulted in severe stock losses in an importing facility in Eritrea, where after its introduction, eradication of the disease required depopulation and fallowing (Lightner et al. 2012b).

Although "*Ca.* H. penaei" has been detected in wild prawns (Aguirre Guzman et al. 2010; Rio Rodríguez et al. 2006; Vazquez-Sauceda et al. 2016), no reports were found about the impact of "*Ca.* H. penaei" on wild prawn populations.

6.2.8 Current biosecurity measures

The Prawn IRA 2009 determined the unrestricted risk associated with "*Ca.* H. penaei" was negligible for frozen product and therefore biosecurity measures were not necessary (Biosecurity Australia 2009).

The Prawn IRA 2009 determined the unrestricted risk associated with "*Ca.* H. penaei" to be moderate for chilled product and therefore biosecurity measures were necessary, including country or zone freedom (Biosecurity Australia 2009).

6.2.9 Conclusion

"*Ca.* H. penaei" is present in exporting countries, is not present in Australia and is capable of causing adverse effects in Australia. In Australia, infection with "*Ca.* H. penaei" is a nationally notifiable disease and biosecurity measures are currently in place for chilled product. Based on the preceding information, risk assessment is warranted.

6.3 Risk assessment

Based on chapter 4 and the technical information about "*Ca.* H. penaei" presented in this chapter, the risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of "*Ca.* H. penaei" meets Australia's ALOP are shown in Appendix F.

6.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for "*Ca.* H. penaei" were that:

- This risk review is generic and therefore the entry assessment assumes that "*Ca.* H. penaei" is present in all source countries.
- "*Ca.* H. penaei" infects penaeid prawn species of marketable size that are exported to Australia.
- Prevalence of "*Ca.* H. penaei" can range from 0–86% in farmed prawns and 0–17% in wild prawn populations.
- "*Ca.* H. penaei" would be present in the prawn head and faeces (gut).
- The load of "*Ca.* H. penaei" in infected imported prawns is likely to be sufficient to cause infection in susceptible species.
- Post-harvest inspection may detect grossly abnormal prawns that are infected with "*Ca.* H. penaei" and remove them before export. Prawns with mild gross signs or which do not show clinical signs would be unlikely to be detected.
- "*Ca.* H. penaei" in imported prawns would not be expected to survive freezing, transport and storage and would be unlikely to be infectious at the time of import.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of "*Ca.* H. penaei" in imported prawns was estimated to be **very low**.

6.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for "*Ca.* H. penaei" were that:

- "*Ca.* H. penaei" would be present in the prawn head (and to a lesser extent the faeces) of infected prawns or in associated wastes that may enter the environment of the exposure groups.
- "*Ca.* H. penaei" would be expected to be present in sufficient loads in imported prawns (or associated wastes) to cause infection in susceptible species if exposed.
- Due to its thermal sensitivity, "*Ca.* H. penaei" is not expected to persist and remain infectious in frozen imported prawns (or associated wastes) at the point of exposure.

- Important aquaculture and wild-caught species in Australia that are susceptible to "*Ca.* H. penaei" infection include *P. monodon* and *P. merguiensis*.
- Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustaceans would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a farm has not implemented standards of entry-level biosecurity for intake water that would exclude "*Ca.* H. penaei" or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.
- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Species susceptible to "*Ca.* H. penaei" may be present in research facilities and public aquaria, but because the host range is relatively narrow this is considered less likely than for hazards with wider host ranges such as WSSV and yellow head virus genotype 1 (YHV1).
- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley by recreational fishers. Prawn species susceptible to "*Ca.* H. penaei" are present in Australian waters and are likely to encounter imported prawns used as bait or berley. The host range for "*Ca.* H. penaei" is narrow compared to hazards such as WSSV or YHV1, therefore the likelihood of exposure is less than for those hazards.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to "*Ca.* H. penaei" in imported prawns was estimated to be:

- Farmed crustaceans—**Extremely low**.
- Hatchery crustaceans—**Extremely low**.
- Wild crustaceans—**Very low**.

6.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to "*Ca.* H. penaei" in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Extremely low**.
- Hatchery crustaceans—**Extremely low**.
- Wild crustaceans—**Extremely low**.

6.3.4 Consequence assessment

Partial likelihood of establishment and spread

The key points considered relevant when determining the partial likelihood of establishment and spread for "*Ca.* H. penaei" were that:

- "*Ca.* H. penaei" can be transmitted horizontally through ingestion of infected tissues, infected faeces or agent in water. Transmission of "*Ca.* H. penaei" from broodstock to progeny may also occur.
- The main prawn species farmed in Australia are susceptible to "*Ca.* H. penaei" infection.
- It is expected that susceptible species feeding on "*Ca.* H. penaei"-infected prawns would receive an infectious dose.
- Prawns that survive "*Ca.* H. penaei" infection can carry infectious "*Ca.* H. penaei" and transmit it to other populations.
- Potential vectors of "*Ca.* H. penaei" are present in Australia and include microalgae, zooplankton and brine shrimp which may aid in spread of "*Ca.* H. penaei".
- The likelihood of "*Ca.* H. penaei" establishment, following a given quantity of "*Ca.* H. penaei" entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.
- If establishment of "*Ca.* H. penaei" were to occur in the wild, spread to other populations would be less likely than for farmed crustaceans because infected wild animals (particularly those clinically affected) are likely to be prey for non-susceptible animals. The densities of susceptible animals are also much lower which reduces the opportunities for transmission. "*Ca.* H. penaei" can remain infectious in recovered animals and vectors such as microalgae, zooplankton and brine shrimp are present in the wild. Therefore "*Ca.* H. penaei" is expected to persist in the environment longer than other hazards. Spread of "*Ca.* H. penaei" to its natural geographical limits is more likely compared to hazards such as Laem-Singh virus.
- If "*Ca*. H. penaei" were to establish in the wild, especially in waters around prawn farms, it may spread to farms due to being transmissible through water. In the absence of effective biosecurity measures, wild infected prawns or vectors such as microalgae, zooplankton and brine shrimp may be transferred into the farms through the inlet water channels. The only known non-prawn species capable of being infected (through experimental challenge-only) with "*Ca*. H. penaei" is *H. americanus* which is not present in Australia.
- If "*Ca*. H. penaei" were to establish on a farm it could spread to neighbouring farms or wild populations through effluent water. This spread would be moderated by dilution effects and the implementation of biosecurity measures should an incursion of "*Ca.* H. penaei" be suspected and response measures initiated. However, "*Ca.* H. penaei" is effectively transmitted through water, and susceptible animals which share a common water source with an infected population may be exposed to "*Ca.* H. penaei".
- Spread from farms to wild populations or neighbouring farms via escaped prawns is possible, although likelihood is reduced due to the systems in place on farms to prevent discharge of live animals.
- If "*Ca.* H. penaei" were to establish in hatchery crustaceans, spread to wild crustaceans would be unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.

- Spread of "*Ca.* H. penaei" from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae as prawn species cultured in Australia are susceptible to infection with "*Ca.* H. penaei". Grossly normal broodstock used in the hatchery could carry infectious "*Ca.* H. penaei" and pass it on to their progeny. Postlarvae may not show clinical signs of disease at the time of transfer to the farm.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of "*Ca.* H. penaei" in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Low**.

Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of "*Ca.* H. penaei" were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- Australia's main farmed prawn species are susceptible to "*Ca.* H. penaei". There is high morbidity and mortality associated with infection in *P. vannamei*. There were no reports about mortality and morbidity in *P. monodon* and *P. merguiensis* infected with "*Ca.* H. penaei" and it is suspected they may not be overly susceptible to significant disease.
- "*Ca.* H. penaei" establishment may affect hatchery prawns as NHP has been reported to cause a reduction in fertility of female broodstock.
- "*Ca.* H. penaei" would not be expected to impact wild fisheries in Australia. There are few reports of "*Ca.* H. penaei" in wild prawns and no reports of declines in catch rates or associated mortalities.
- Based on the impacts in the Americas from "*Ca.* H. penaei" infection, "*Ca.* H. penaei" establishment and spread in Australia would be expected to cause minor impacts at the state or territory level on the life or health of susceptible animals.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There are no known effects on the living environment—there are few reports of "*Ca.* H. penaei" in wild prawns and there are no reports of mortalities.
- "*Ca.* H. penaei" has been detected in *Artemia franciscana, Navicula* species and zooplankton. Whilst these species are found in Australia they are proposed to act as vectors in the environment where "*Ca.* H. penaei" occurs in susceptible species, rather than being a susceptible species *per se*.
- The direct impact of "*Ca.* H. penaei" establishment and spread on the environment is not expected to be discernible at any level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- NHP is listed as a notifiable disease by the WOAH and is included on Australia's *National list of reportable diseases of aquatic animals.* State and territory governments would be expected to report on the agent.
- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating "*Ca.* H. penaei" from wild crustacean populations is unlikely to be launched.
- If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely.
- If a movement regulated area were put in place for an outbreak of "*Ca.* H. penaei", there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.
- Eradication of "*Ca.* H. penaei" is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted, or decreased feed companies would be impacted by reduced feed purchases.
- "*Ca.* H. penaei" affected prawns would likely show gross signs which may affect their marketability.
- "*Ca.* H. penaei" establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- "*Ca.* H. penaei" is a WOAH -listed disease. Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. "*Ca.* H. penaei" establishment and spread may result in loss of some crustacean export markets due to importing country biosecurity requirements.
- If "*Ca.* H. penaei" were to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product.
- The impacts of "*Ca.* H. penaei" establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- No endangered Australian crustacean species, or closely related species, are currently considered susceptible to "*Ca.* H. penaei".
- The impacts of "*Ca.* H. penaei" establishment and spread on biodiversity are not expected to be discernible at any level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Prawns that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of "*Ca.* H. penaei" which may impact on social amenity.
- The social impacts of "*Ca.* H. penaei" establishment and spread are expected to be minor at the local level.

Table 12 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of "*Ca.* H. penaei". The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 12 Overall impact of establishment and spread of "Ca. H. penaei" for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	State or territory	Minor	D
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	A
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	B

Conclusion
The overall impact of establishment and spread of "*Ca.* H. penaei" was estimated to be **moderate**.

Determination of likely consequences for outbreak scenario
The likely consequences of the outbreak scenario for "*Ca.* H. penaei" in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Low**.

6.3.5 Determination of partial annual risk

The partial annual risk of "*Ca.* H. penaei" entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Negligible**.
- Hatchery crustaceans—**Negligible**.
- Wild crustaceans—**Negligible**.

6.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with "*Ca.* H. penaei" in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **negligible**.

Therefore, as the overall annual risk achieves Australia's ALOP, specific biosecurity measures are not considered necessary for this hazard when product is imported frozen.

Because freezing was considered to be the critical factor in this product achieving Australia's ALOP, the overall annual risk was also estimated for chilled, uncooked, whole prawns intended for human consumption and found to be **low** (the risk assessment values for chilled product are shown in Appendix F).

Therefore, the overall annual risk for chilled, uncooked, whole prawns intended for human consumption does not achieve Australia's ALOP. Biosecurity measures, other than country, compartment or zone freedom have not been assessed since importation of uncooked, chilled product is generally unfeasible. A submission can be made to the department by any parties interested in exporting uncooked chilled product to Australia.

7 Covert mortality nodavirus risk review

7.1 Background

Covert mortality nodavirus (CMNV) is the aetiological agent of viral covert mortality disease (VCMD) (Zhang et al. 2014; Zhang et al. 2017b). VCMD was so named because affected prawns died at the bottom of the pond instead of at the surfaces or edges and farmers would initially be unaware of the mortality (Zhang et al. 2014). CMNV is a member of the *Nodaviridae* family (Zhang et al. 2014). Both penaeid and caridean prawn species as well as some finfish species are susceptible to infection with CMNV (Zhang et al. 2014; Zhang et al. 2017b).

CMNV is reported to have caused mortalities in penaeid prawns in China since 2002–2003 (Zhang et al. 2014; Zhang et al. 2017b). CMNV has been detected throughout Asia and in Ecuador and Mexico (Flegel 2015a; NACA 2018; Pooljun et al. 2016; Thitamadee et al. 2016; Zhang et al. 2017b).

Infection with CMNV is not listed as a disease notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c) nor is it included in Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Infection with CMNV is included in the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2019a). Australia has a long history of passive surveillance and a strong system in place to detect incursions. CMNV is considered exotic to Australia.

7.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of CMNV is warranted.

7.2.1 Agent properties

CMNV is a spherical, non-enveloped, single-stranded, positive sense RNA virus approximately 32nm in diameter (Zhang et al. 2014; Zhang et al. 2017b). Phylogenetic analysis of the RNA-dependent RNA polymerase gene of CMNV classifies the nodavirus as a member of the genus *Alphanodavirus*, in the family *Nodaviridae* (Xu et al. 2020; Zhang et al. 2014).

There are no reports on the stability of CMNV. However, stability information is available for other members of the genus *Alphanodavirus*, such as *Penaeus vannamei* nodavirus (PvNV) and the closely related *Macrobrachium rosenbergii* nodavirus (MrNV). PvNV can survive freezing at −70°C (Tang et al. 2007b). MrNV can survive freezing at −20°C and is inactivated by heat treatment at 50°C for at least 5 mins (Ravi & Sahul Hameed 2016).

7.2.2 Epidemiology

Host range

Species which are susceptible to infection (N= natural; E= experimental exposure) with CMNV include:

- *Apostichopus japonicas* [N] (sea cucumber)(Wang et al. 2021c)
- *Carassius auratus* [N] (finfish) (Wang et al. 2019)

- *Corophium sinense Zhang* [N] (amphipod) (Liu et al. 2018b)
- *Danio rerio* [N, E] (finfish) (Wang et al. 2021a; Wang et al. 2022)
- *Diogenes edwardsii* [N] (hermit crab) (Liu et al. 2018b)
- *Exopalaemon carinicauda* [N, E] (Liu et al. 2017)
- *Larimicthys polyactis* [N] (finfish) (Xu et al. 2021a)
- *Macrobrachium rosenbergii* [N] (Zhang et al. 2017b)
- *Mugilogobius abei* [N] (finfish) (Zhang et al. 2018)
- *Ocypode cordimundus* [N] (ghost crab) (Liu et al. 2018b)
- *Paralichthys olivaceus* [N] (finfish) (Wang et al. 2018)
- *Parathemisto gaudichaudi* [N] (amphipod) (Liu et al. 2018b)
- *Penaeus chinensis* [N] (Zhang et al. 2014; Zhang et al. 2017b)
- *Penaeus japonicus* [N] (Zhang et al. 2014; Zhang et al. 2017b)
- *Penaeus monodon* [N] (Zhang et al. 2017b)
- *Penaeus vannamei* [N, E] (Thitamadee et al. 2016; Zhang et al. 2014; Zhang et al. 2017b)
- *Tubuca arcuate* [N] (fiddler crab) (Liu et al. 2018b).

Species for which CMNV-positive PCR results (nested RT-PCR) and/or CMNV-positive results by reverse transcription loop mediated isothermal amplification (RT-LAMP) have also been reported (N= natural; E= experimental exposure) include:

- *Artemia sinica* [N] (Liu et al. 2018b)
- *Balanus* species [N] (barnacle) (Liu et al. 2018b)
- *Brachionus urceus* [N] (rotifer) (Liu et al. 2018b)
- *Chaeturichthys hexanema* [N] (finfish) (Zhang et al. 2018)
- *Crassostrea gigas* [N] (Pacific oyster) (Liu et al. 2018b)
- *Meretrix lusoria* [N] (common clam) (Liu et al. 2018b)
- unidentified gammarid amphipod [N] (Liu et al. 2018b).

C. gigas, A. sinica and Balanus species. are considered likely vectors of CMNV as infection was not confirmed (Liu et al. 2018b). Additionally, bivalve molluscs are well known to be successful bioaccumulators of viruses from the environment (Burge et al. 2016).

CMNV has been detected in multiple prawn life stages, including nauplii, postlarvae, juveniles and broodstock (Huang 2015).

Geographical distribution

Covert mortality disease was initially observed in farming ponds of *P. vannamei* in China before 2009, but not until 2014 was CMNV proven to be the infectious agent of the disease and the disease renamed as VCMD (Zhang et al. 2004 and Xing et al. 2004 cited in (Zhang et al. 2014; Zhang et al. 2017b)). CMNV has since been detected in other Asian countries including India (Flegel 2014), Thailand (Pooljun et al. 2016; Thitamadee et al. 2016) and Vietnam (Zhang et al. 2017b). In addition, CMNV has been detected in *P. vannamei* in Mexico (Huang 2015) and Ecuador (Zhang et al. 2017b).

Prevalence

The prevalence rates of CMNV among 843 farmed prawn samples collected from 145 sampling sites located in 10 provinces in China in 2013, 2014 and 2015 were 46% (130/283), 28% (84/301) and 21% (54/259), respectively (Zhang et al. 2017b). The same prevalence study reported CMNV was found in 60% (9/15) of *P. japonicus*, 33% (228/694) of *P. vannamei*, 33% (3/9) of *P. monodon,* 24% (9/37) of *M. rosenbergii* and 22% (19/88) of *P. chinensis* samples (Zhang et al. 2017b). A study on the prevalence of CMNV in farmed *E. carinicauda* from China detected the virus in 27% of prawn samples (sample numbers not reported) (Liu et al. 2017). In an epidemiological survey conducted on prawn ponds in Thailand, 148 prawn samples were collected and CMNV detected in 43% (64/148) (Flegel 2015a). In a separate study conducted on 69 prawn samples collected from prawn farms in 4 southern provinces in Thailand, CMNV was detected at a prevalence of 37% (26/69) (Pooljun et al. 2016).

In China, a CMNV prevalence of 39% (7/18) was reported in a population of *M. abei* finfish collected from prawn ponds suffering VCMD and from surrounding coastal waters near the drainage channel of the farm (Zhang et al. 2018). In an epidemiological survey of wild fish, samples of *L. polyactis* were collected from the Yellow Sea and East China Sea in 2018 and 2019. The CMNV prevalence was found to be 18% and 7%, respectively (Xu et al. 2021a). Another epidemiological investigation detected CMNV in 6.56% (4/61) samples of sea cucumbers *Apostichopus japonicus*, collected from polyculture ponds of prawns and sea cucumber in China (Wang et al. 2021b).

It has been reported that CMNV is often associated with co-infections with white spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND) (Flegel 2015a).

Mortalities

CMNV is reported to have caused losses in China since before 2009 (Zhang et al. 2014). Prawn farmers reported that moribund and dead prawns could be found every day in diseased ponds. The mortality began 1 month post-stocking and increased after 60–80 days post-stocking with a cumulative mortality up to 80% (Zhang et al. 2014). However, there have also been reports that VCMD can occur as early as 1–2 weeks post-stocking (Zhang et al. 2017b). In an experimental challenge infection, 100% mortality was observed in *P. vannamei* injected with CMNV-positive tissue homogenate 10 days post-infection and 85% mortality occurred in prawns fed CMNV-positive tissue (Zhang et al. 2014). Mortality due to CMNV appears to be exacerbated by a sudden change in environmental conditions, such as high nitrite levels and high temperature (>28°C) (Zhang et al. 2014; Zhang et al. 2017b).

Mortalities have also been detected in CMNV-infected fish. *D. rerio* injected with 10 µL CMNV (5.6×10^5 copies/µl) resulted in cumulative mortalities of up to 53.33% within 14 days post–infection (Wang et al. 2022).

Transmission

CMNV can be transmitted horizontally by the ingestion of infected tissues (Thitamadee et al. 2016; Zhang et al. 2014). Experimental infections have also been induced by injection of homogenised infected tissues (cephalothoraxes and white abdominal muscle) (Thitamadee et al. 2016; Zhang et al. 2014).

Transmission of CMNV from broodstock to progeny was demonstrated in *E. carinicauda*, where CMNV virions were observed in oogonia, oocytes, spermatocytes, fertilized eggs and nauplii (Liu et al. 2017). The results suggest *E. carinicauda* may be one of the main hosts of CMNV (Liu et al. 2017). A wide range of other possible hosts for CMNV have been identified, including, *C. sinense zhang, D. edwardsii, O. cordimanus, P. gaudichalldi* and *T. arcuata* (Liu et al. 2018b). The *in situ* hybridisation assay (ISH) in this study confirmed CMNV infection in those five species, indicating they can be considered susceptible species (Liu et al. 2018b). Other possible vectors include *C. gigas, A. sinica and Balanus* species (Liu et al. 2018b).

Samples of *M. abei* collected from CMNV-infected *P. vannamei* ponds and surrounding coastal waters in China, tested CMNV-positive by RT-LAMP assay and ISH (Zhang et al. 2018). CMNV was similarly detected by RT-LAMP in *P. olivaceus*, a farmed Japanese flounder that shared facilities with CMNV-positive farmed *P. vannamei* (Wang et al. 2018). CMNV was also identified (RT-LAMP and RT-PCR) in nearshore *C. hexanema*, another wild marine fish in the Yellow Sea (Zhang et al. 2018). Together, these results suggest that cross-species transmission can occur at the level of Phyla and that CMNV may be transmitted by cohabitation with infected fish and possibly by water (Wang et al. 2018; Zhang et al. 2018).

Mechanism of spread

The mechanism of CMNV spread into new countries and/or areas has not been determined. The introduction of CMNV into new areas is likely attributed to the movement of live animals. It has been reported that CMNV can be transmitted from broodstock to progeny in *E. carinicauda* (Liu et al. 2017).

Infectious dose

The minimum infectious dose of CMNV required to cause VCMD in susceptible species by experimental challenge or natural infection is not known. However, *per os* infection of *P. vannamei* fed with minced CMNV-infected tissues (9 mm^3) at 10% of total body weight resulted in cumulative mortality of 84.85 ± 2.14% at 10 days post-infection (Zhang et al. 2014). In the same study, 100% mortalities were observed in *P. vannamei* following injection of a CMNV homogenised inoculum prepared from cephalothoraxes and whitish abdominal muscle (Zhang et al. 2014).

7.2.3 Pathogenesis

Tissue tropism

In prawns, CMNV infects the hepatopancreas, striated muscle and lymphoid organ (Zhang et al. 2014). CMNV has also been detected in oogonia, oocytes, spermatocytes and fertilized eggs of experimentally infected *E. carinicauda* broodstock (Liu et al. 2017).

In finfish, CMNV infects muscle and oocytes as well as the eye, gills, brain, kidneys, spleen, and intestines (Wang et al. 2018; Wang et al. 2021a; Wang et al. 2022; Wang et al. 2019; Xu et al. 2021a; Zhang et al. 2018). In sea cucumbers, intestine, respiratory trees, testis and gonad are infected by CMNV (Wang et al. 2021b).

Tissue titre

One study that examined the titre of CMNV in infected *P. vannamei* found that the viral loads varied from 1.5×10^2–6.7×10^6 copies/mg of cephalothorax tissue when examined by real-time RT-LAMP (Zhang et al. 2017a). Pooljun et al. (2016) similarly showed the viral load in CMNV-infected prawn

samples from Thailand varied from 4.3–6.5 × 10^6 copies/µL of total RNA when analysed by qRT-PCR (Pooljun et al. 2016). The viral load in the muscles of CMNV infected *M. abei* varied from 4.9–3.5 × 10^4 copies/mg tissue (examined by real-time RT-LAMP), which was lower than the viral load in the muscles of *P. vannamei* (2.1 × 10^1–8.3 × 10^5 copies/mg tissue) (Zhang et al. 2018).

7.2.4 Diagnosis

Clinical signs

Prawns infected with CMNV exhibit hepatopancreatic atrophy and necrosis, empty stomach and guts, soft shell, slow growth, and in many cases abdominal muscle whitening (Zhang et al. 2014). These clinical signs are similar to those caused by other pathogenic agents such as infectious myonecrosis virus or seen in prawns with AHPND, making diagnosis based on clinical signs difficult (Zhang et al. 2014).

Finfish infected with CMNV may appear grossly normal whilst others show signs of stunted growth, eyeball enlargement or abnormal swimming behaviour (Wang et al. 2018; Zhang et al. 2018). It is unknown if crabs develop clinical signs following infection with CMNV.

Pathology

Histopathological examination of prawns suffering VCMD revealed coagulative necrosis of striated muscle accompanied by haemocytic infiltration and karyopyknosis of haemocyte nuclei (Zhang et al. 2014). Additionally, eosinophilic inclusions were found in the tubular epithelium of the hepatopancreas and lymphoid organ, and mass karyopyknotic nuclei were detected in the muscle and lymphoid organ (Zhang et al. 2014; Zhang et al. 2017b). Enlarged nuclei in the hepatopancreas have also been observed (Thitamadee et al. 2016).

Histopathological analysis revealed CMNV infection in fish species *M. abei, C. auratus, L. polyactis* and *P. olivaceus* could cause extensive necrosis of skeletal and cardiac muscle and nervous tissue vacuolation in the eye and brain (Wang et al. 2018; Wang et al. 2019; Xu et al. 2021a; Zhang et al. 2018). CMNV-positive *L. polyactis* also exhibited oocytes loosely arranging, degenerate renal tubular cells and a reduction in spleen cells (Xu et al. 2021a). Spleen and kidney of infected *P. olivaceus* also presented cell necrosis, degeneration and karyopyknosis (Wang et al. 2018). In CMNV-infected *D. rerio*, gill tissues became disordered and distorted and the intestines showed karyopyknosis with infiltrated lymphocytes (Wang et al. 2022).

Histopathological lesions have also been reported in intestine, respiratory trees, testis and gonad of sea cucumber *A. japonicus*. The histopathological lesions of the diseased sea cucumber showed the pathological changes of CMNV infection similar to histopathological lesions in prawns and fish infected with CMNV (Xu et al. 2021a).

Testing

Nested RT-PCR, qRT-PCR and RT-LAMP targeting RNA-dependent RNA polymerase are methods used to detect CMNV (Li et al. 2018; Pooljun et al. 2016; Zhang et al. 2014; Zhang et al. 2017a). ISH can also be used to screen for CMNV (Zhang et al. 2014; Zhang et al. 2017b).

7.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments.

7.2.6 Control

Control measures for CMNV are primarily aimed at screening for CMNV in broodstock before spawning and in postlarvae before stocking ponds to help reduce disease prevalence and dissemination (Liu et al. 2017). Specific pathogen-free *P. vannamei* stocks have been developed for pathogens, including CMNV (Kona Bay 2020; Muhammad 2017). The potential for vertebrate and invertebrate species in and around prawn ponds to be vectors or susceptible hosts of CMNV shows that special attention should be paid to pond disinfection before stocking and that live or fresh feed should be either pre-screened for CMNV or not used (Liu et al. 2018b).

7.2.7 Impact of the disease

Infection with CMNV has caused cumulative mortalities of up to 80% in farmed prawns. Although CMNV has been reported to cause significant economic losses to the prawn aquaculture industry (Liu et al. 2018b; Zhang et al. 2017b), no reports were found detailing the production or market costs of infection with CMNV.

Although, CMNV has been reported in wild finfish collected from the Yellow Sea and East China Sea (Xu et al. 2021a) and surrounding coastal waters near the drainage channel of prawn farms (Wang et al. 2019; Zhang et al. 2018), no reports were found about the impact of CMNV on wild crustacean or finfish populations.

7.2.8 Current biosecurity measures

CMNV was not assessed in the Prawn IRA 2009 and there are no current biosecurity measures specific for CMNV.

7.2.9 Conclusion

CMNV is present in exporting countries, is not present in Australia and is capable of causing adverse effects. In Australia, infection with CMNV is not a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

There are reports of CMNV infecting finfish species. The two finfish species which are reported in Australia (*C. auratus* and *D. rerio*) have been considered in this risk assessment. It is acknowledged that there are species related to CMNV susceptible finfish at the genus level or higher which are native to Australia (for example, genus *Mugilogobius* (Fishes of Australia 2015a), family Paralichthyidae (Fishes of Australia 2015b) and family Gobiidae (Bray 2017)). However, the review follows the criteria in Article 1.5.9. of the WOAH *Aquatic animal health code* (WOAH 2022c) for listing susceptible species at a ranking of genus or higher. None of the CMNV susceptible finfish species meet this criterion. Should information become available that suggests finfish species native to Australia are susceptible to CMNV, the department will reconsider the risk assessment for CMNV.

7.3 Risk assessment

Based on chapter 4 and the technical information about CMNV presented in this chapter, a risk assessment was completed. The definition of the exposure groups in this risk assessment is different to the other risk assessments. This is to take into account the susceptibility of finfish species in Australia to CMNV. The exposure groups considered in the CMNV risk assessment are:

- farmed susceptible species

- hatchery susceptible species (encompassing hatchery broodstock and postlarvae as well as susceptible species in research facilities, ornamental finfish facilities, public aquaria and private aquaria)
- wild susceptible species.

A summary of the risk assessment values for determining if the overall annual risk of CMNV meets Australia's ALOP are shown in Appendix F.

7.3.1 Entry assessment

The key points were considered relevant when conducting the entry assessment for CMNV were that:

- This risk review is generic and therefore the entry assessment assumes that CMNV is present in all source countries.
- CMNV infects various penaeid and caridean prawn species of marketable size that are exported to Australia.
- Prevalence of CMNV ranges from 21–46% in farmed prawns. There are no reports of CMNV prevalence in wild prawns. However, CMNV has been reported at 7–39% prevalence in the wild marine fish collected in and around infected prawn ponds.
- CMNV would be present in the whole body of infected prawns.
- The viral load of CMNV in infected imported prawns is likely to be sufficient to cause infection in susceptible species.
- Post-harvest inspection may detect grossly abnormal prawns that are CMNV-positive and remove them before export. Prawns with mild gross signs or which do not show clinical signs would be unlikely to be detected.
- It is assumed that CMNV in imported prawns would survive freezing, storage and transport and remain infectious at the time of import.
- The risk of CMNV entry through imported finfish is not considered as it is outside the scope of this review. The biosecurity risks associated with the import of live ornamental fish and finfish for human consumption are considered in separate policies.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of CMNV in imported prawns was estimated to be **high**.

7.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for CMNV were that:

- CMNV would be present in the whole body of infected prawns or in associated wastes that may enter the environment of the exposure groups.
- CMNV would be expected to be present in sufficient loads in imported prawns (or associated wastes) to cause infection in susceptible species if exposed.
- CMNV in imported prawns (or associated wastes) is likely to persist and remain infectious at the point of exposure.
- Important aquaculture and wild-caught species in Australia, that are susceptible to CMNV infection include, *P. monodon, P. japonicus* and *M. rosenbergii*. Other CMNV susceptible species

and potential vectors are widespread in Australian waters including some crabs, brine shrimp, oysters and barnacles.

- CMNV can infect *C. auratus* and *D. rerio*, which are freshwater finfish present in Australia.
- Farmed susceptible species are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery susceptible species would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed susceptible species are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a farm has not implemented standards of entry-level biosecurity for intake water that would exclude CMNV or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.
- Susceptible species present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume of whole uncooked prawns would be used as feed for susceptible species in public aquaria and research facilities. Species susceptible to CMNV are likely to be present in research facilities and public aquaria.
- Wild susceptible species would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, susceptible species must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild susceptible species are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley by recreational fishers and due to the wide host range of CMNV.
- *C. auratus* are farmed in some locations within Australia but the facilities and production are not large due to the expense in producing the finfish, and the price they can be sourced from overseas (Department of Agriculture 2014b). *C. auratus* farms are considered within the hatchery exposure group because of the expected physical containment requirements of the facilities. *D. rerio* are not farmed within Australia. Both *C. auratus* and *D. rerio* are traded in the ornamental fish industry so are present in ornamental finfish facilities including wholesalers, hatcheries and in public and private aquaria. *D. rerio* is frequently used as an experimental animal model and is commonly found in research institutions. There is likely to be breeding of *D. rerio* within research institutions. It was considered very unlikely that these finfish would be feed with imported prawns due to the size and nutritional requirements of these finfish species. Therefore, they were not considered to contribute to the overall exposure likelihood rating for farmed and hatchery exposure groups. *C. auratus* is found in the wild in Australia so may be directly exposed to imported prawns used as bait and berley. It is unknown, if they would or could consume a sufficient dose of CMNV from a prawn used as bait to become infected. There are no reports of *D. rerio* being present in the wild in Australia.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to CMNV in imported prawns was estimated to be:

- Farmed susceptible species—**Very low**.
- Hatchery susceptible species—**Low**.
- Wild susceptible species—**Moderate**.

7.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to CMNV in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed susceptible species—**Very low**.
- Hatchery susceptible species—**Low**.
- Wild susceptible species—**Moderate**.

7.3.4 Consequence assessment

Partial likelihood of establishment and spread

The key points considered relevant when determining the partial likelihood of establishment and spread for CMNV were that:

- CMNV can be transmitted by ingestion of infected tissues, cohabitation, water and from broodstock to progeny.
- It is expected that susceptible species feeding on CMNV-infected prawns would receive an infectious dose.
- It is unknown if prawns that survive CMNV infection can remain infectious.
- CMNV susceptible species and potential vectors are present in Australia and include crabs, brine shrimp, finfish, barnacles and oysters.
- Important aquaculture and wild-caught species in Australia that are susceptible to CMNV infection, include *P. monodon, P. japonicus* and *M. rosenbergii*.
- CMNV can infect *C. auratus* and *D. rerio*, freshwater finfish present in Australia.
- The likelihood of CMNV establishment, following a given quantity of CMNV entering the environment of an exposure group, is the greatest for farmed susceptible species. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.
- If establishment of CMNV were to occur in the wild, spread to other populations would be less likely than for farmed or hatchery susceptible species because infected wild animals (particularly those clinically affected) are likely to be prey for non-susceptible animals. The densities of susceptible animals are also much lower which reduces the opportunities for transmission. The host range of CMNV present in Australia is smaller than for other hazards such as WSSV which also reduces the opportunities for transmission and spread to its natural geographic limits.
- Establishment and spread of CMNV due to susceptible finfish species *C. auratus* and *D. rerio* is not considered to appreciably contribute to the partial likelihood of establishment and spread for CMNV. This is because:
  - if CMNV were to establish in *C. auratus* and *D. rerio*, spread to prawn farms and hatcheries is unlikely as these species are not expected to be present around prawn farms. This is because *P. monodon* grow under a wide range of salinities with maximum growth rates occurring in 15-20

parts per thousand (ppt) salinity, whereas *C. auratus* and *D. rerio* are freshwater species. *C. auratus* can tolerate low levels of salinity (up to and including 10ppt) but higher salinity levels of 20ppt cause significant adverse effects on health and even death in some cases (küçük 2013; Schofield, Brown & Fuller 2006). *D. rerio* are not reported in the wild in Australia. *D. rerio* also have a very limited salinity tolerance of 0.1- 0.6ppt (Lawrence 2007).

- Establishment and spread of CMNV to the wild from hatchery populations of these finfish species would be unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.
- It is unknown if CMNV could spread from hatchery to farm via finfish.

- Establishment and spread of CMNV due to susceptible crustacean species is considered to significantly contribute to the partial likelihood of establishment and spread for CMNV. This is because:
  - If CMNV were to establish in the wild, especially in waters around prawn farms, it may easily spread to farms due to being transmissible through water. In the absence of effective biosecurity measures, wild infected prawns may be transferred into the farms through the inlet water channels along with potential vectors such as *Artemia*. There are species known to be susceptible to infection with CMNV, for example the crab *O. cordimundus*, which are present in Australia and may be capable of entering farms through movement across short distances of land.
  - If CMNV were to establish on a farm it could spread to neighbouring farms or wild populations through effluent water. This spread may be moderated by dilution effects and implementation of biosecurity measures should an incursion of CMNV be suspected and response measures initiated. However, CMNV is effectively transmitted through water, and susceptible animals which share a common water source with an infected population may be exposed to CMNV. Although it is unknown how long CMNV can persist in the water column without a host and remain infectious.
  - Spread from farms to wild populations or neighbouring farms via escaped prawns is possible, although likelihood is reduced due to the systems in place on farms to prevent discharge of live animals.
  - If CMNV were to establish in hatchery crustaceans, spread to the wild would be unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.
  - Spread of CMNV from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae as prawn species cultured in Australia are susceptible to infection with CMNV. CMNV has been demonstrated to be transferred from broodstock to progeny and postlarvae do not show clinical signs of infection until after transfer to the farm.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of CMNV in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed susceptible species—**Moderate**.
- Hatchery susceptible species—**Low**.
- Wild susceptible species—**Very low**.

Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of CMNV were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- Australia's main farmed prawn species, *P. monodon* is susceptible to CMNV. There is high morbidity and mortality associated with infection.
- CMNV would not be expected to impact wild fisheries in Australia. There are reports of CMNV in wild finfish but no reports of CMNV in wild prawns. There is no evidence of declines in wild catch rates or associated mortalities due to CMNV.
- Significant impacts have been reported in CMNV affected prawn farms in China. Based on the limited reports of the impact of CMNV infection, CMNV establishment and spread in Australia would be expected to have minor impacts at the national level on the life or health of susceptible animals.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There are no reports on the impacts of CMNV on the living environment.
- Prawns, finfish and other aquatic species are known to be susceptible to CMNV. If CMNV infection spreads to native marine finfish or crabs it could cause mortalities in these wild populations.
- The direct impact of CMNV establishment and spread on the environment is not expected to be discernible at any level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with CMNV is not listed as a notifiable disease by the WOAH but it is included in the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2019a). CMNV is not included on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Although not listed in Australia, state and territory governments would be expected to report on the presence of an unlisted agent that has never been reported in Australia.
- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating CMNV from wild susceptible species is unlikely to be launched.
- If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely.
- If a movement regulated area were put in place for an outbreak of CMNV, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.
- Eradication of CMNV is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Movement regulated areas put in place would have indirect impacts on other industries such as seafood suppliers, ornamental fish facilities and commercial wild catch fisheries due to the broad host range of CMNV.

- Industries supplying inputs into the affected regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- CMNV affected prawns would likely show gross signs which may affect their marketability.
- CMNV establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. CMNV establishment and spread may result in loss of some crustacean export markets.
- Internationally, there are no known import requirements for CMNV in live freshwater species *C. auratus* or *D. rerio*. Export of any freshwater ornamental finfish is therefore not expected to be affected in the event of a CMNV outbreak.
- If CMNV was to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product.
- The impacts of CMNV establishment and spread on international trade are likely to be minor at the local level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- CMNV has a small host range of species present in Australia which are known to be susceptible to CMNV infection.
- There are no species listed as endangered in Australia that are related to species known to be susceptible to CMNV.
- Movement regulated areas could also affect species *C. auratus* or *D. rerio* in ornamental finfish facilities.
- The impacts of CMNV establishment and spread on biodiversity are not expected to be discernible at any level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Prawns that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of CMNV which may impact on social amenity.
- The social impacts of CMNV establishment and spread are expected to be minor at the local level.

Table 13 shows the individual impact scores for each criteria (determined using Figure 4) for the establishment and spread of CMNV. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 13 Overall impact of establishment and spread of CMNV for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Minor	E
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	Local	Minor	B
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	A
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	B

Conclusion
The overall impact of establishment and spread of CMNV was estimated to be **moderate**.

Determination of likely consequences of the outbreak scenario
The likely consequences of the outbreak scenario for CMNV in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed susceptible species —**Moderate**.
- Hatchery susceptible species —**Low**.
- Wild susceptible species —**Very low**.

7.3.5 Determination of the partial annual risk

The partial annual risk of CMNV entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed susceptible species —**Very low**.
- Hatchery susceptible species —**Very low**.
- Wild susceptible species —**Very low**.

7.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with CMNV in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **low**.

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

7.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for CMNV in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 7.4.1, 7.4.2 and 7.4.3 present the factors considered and the conclusions reached.

7.4.1 Head and shell removal

When determining if head and shell removal would reduce the overall risk of CMNV to meet Australia's ALOP, the key points considered were:

- Head and shell removal is not expected to reduce the likelihood of entry of CMNV because sufficient CMNV to infect susceptible species would still be present in the tail.
- CMNV was not detected in deveined, de-headed and de-shelled prawns during monitoring programs undertaken during 2020–22. These programs included batches from countries where CMNV is endemic. Because CMNV was not detected in these programs, the entry likelihood for CMNV in these products is reduced based on the known approach rates of CMNV.
- Ongoing monitoring (see section 17.4.2 Ongoing monitoring of batches of imported uncooked prawns) of imported batches for CMNV is continuing. This will ensure that any changes to the approach rates of CMNV (and therefore the entry likelihood for these products) is known and additional biosecurity measures can be applied, as necessary.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Head and shell removal is expected to further reduce the likelihood of this occurring because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed for aquatic animals held in research or public aquaria.
- Whole uncooked prawns are preferred over other forms of prawns (including uncooked and shelled) for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal applied was determined to be **negligible.**

7.4.2 Cooking

When determining if cooking would reduce the overall risk of CMNV to meet Australia's ALOP, the key points considered were:

- No reported investigations into the stability of CMNV to heat treatments were found. The closely related MrNV is inactivated by heat treatment at 50°C for at least 5 mins (Ravi & Sahul Hameed 2016).
- Given the uncertainty regarding the effect of heating on CMNV infectivity, it is assumed that cooking may reduce, but not completely inactivate CMNV. Some infectious virus may remain.

Therefore, cooking to attain a core temperature of at least 65°C is expected to reduce the likelihood of entry, but not completely remove it.

- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **negligible**.

7.4.3 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of CMNV to meet Australia's ALOP, the key points considered were:

- Value-added products (VAP) are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). VAP were not sampled during the monitoring programs undertaken in 2020–22 and therefore there are no known approach rates for CMNV in this product.
- Therefore, without knowledge of approach rates, it is considered that head and shell removal and processing into a VAP would not reduce the likelihood of entry of CMNV in the same manner as it was reduced for products which have had the head and shell removed (refer section 7.4.1 Head and shell removal). Whilst head and shell removal would be expected to reduce the viral load in the prawn, sufficient CMNV to cause infection in a susceptible species following exposure is expected to remain. It is also not expected that processing into a VAP will reduce the amount of viable CMNV in the product.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

Conclusion
Based on this information, the overall restricted risk of imported frozen uncooked prawns which have been processed into a value-added product, was determined to be **negligible**.

8 Decapod iridescent virus 1 risk review

8.1 Background

Infection with decapod iridescent virus 1 (DIV1) is a serious emerging disease that causes infection and mortality in farmed *Penaeus vannamei* (Qiu et al. 2017; Qiu et al. 2018a). SHIV (shrimp hemocyte iridescent virus) and CQIV (*Cherax quadricarinatus* iridovirus) were identified separately and are considered to represent two different isolates of DIV1. DIV1 was formally classified by the International Committee on Taxonomy of Viruses (ICTV) in the family *Iridoviridae* (ICTV 2018). Infection with DIV1 has also been referred to as 'white head' or 'white spot' in some publications (Qiu et al. 2019a). Host species susceptible to DIV1 include some penaeid and caridean prawns, as well as crayfish (Qiu et al. 2017; Qiu et al. 2018a; Qiu et al. 2019a).

Infection with DIV1 has been reported in China (Li, Xu & Yang 2017; Qiu et al. 2017; Qiu et al. 2018a; Xu et al. 2016) and Taiwan (OIE 2020b). DIV1 has been detected by PCR in grossly normal wild prawns caught from the Indian Ocean (Srisala et al. 2020a).

Infection with DIV1 is listed as a disease notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c) and is on the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2020c) and on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Australia has a long history of passive surveillance and a strong system in place to detect incursions, DIV1 is considered exotic to Australia.

To simplify naming of the hazard in this chapter, SHIV and CQIV will be referred to as DIV1, unless differentiation is required for clarity.

8.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of DIV1 is warranted.

8.2.1 Agent properties

DIV1 is an icosahedral, double-stranded DNA virus with a mean diameter of around 150nm (Qiu et al. 2017; Qiu et al. 2018b; Xu et al. 2016). DIV1 is classified by the ICTV as a member of the genus *Decapodiridovirus*, in the family *Iridoviridae* (ICTV 2018; Qiu et al. 2017; Qiu et al. 2018b; Qiu et al. 2019a). DIV1 was originally isolated from different hosts and independently described as SHIV and CQIV. Complete genome sequencing has since shown that SHIV and CQIV are 99% identical and that their genome size (approximately 150 kb) and GC content (approximately 35%) are nearly the same (Li, Xu & Yang 2017; Qiu et al. 2018a; Qiu et al. 2018b; Xu et al. 2016).

Phylogenetic analyses using amino acid sequences for two highly conserved genes, major capsid protein (MCP) and ATPase, showed that these DIV1 genes had identities ranging from 46–52% with known members of *Iridoviridae* (Qiu et al. 2017). Specifically, 46%, 46% and 45% identities to those of the MCP from *Armadillidium vulgare* iridescent virus, invertebrate iridescent virus 6 (IIV6) and lymphocystis disease virus 1 (LDV1), respectively. Identities of 52%, 51%, and 51% with those of the

ATPase from LDV1, epizootic haematopoietic necrosis virus (EHNV) and lymphocystis disease virus-isolate China, respectively were reported (Qiu et al. 2017). A NCBI BLAST analysis identified the 34 amino acid MCP sequence to have 55% identity with the MCP of sergestid iridovirus (Xu et al. 2010) which has caused disease in *Acetes erythraeus* (Tang et al. 2007a). *Iridoviridae* is a poorly understood family as it comprises a very large and diverse group of viruses without a clear criteria for identification (Ince et al. 2018). *Iridoviridae* infects a diverse host range that includes invertebrate and vertebrates (Ince et al. 2018). In crustaceans, five iridoviruses have been reported (Lightner & Redman 1993; Montanie, Bonami & Comps 1993; Piegu et al. 2014; Tang et al. 2007a; Xu et al. 2016).

It is likely that DIV1 survives freezing at –80°C as frozen DIV1-positive prawn tissue fed to healthy prawns transmitted the virus (Qiu 2022; Qiu et al. 2017; Qiu et al. 2018a). No other reports on the stability of DIV1 were found.

IIV6 from the rice stem borer, is thermolabile and rapidly inactivated at temperatures above 55°C (Ince et al. 2018), with complete inactivation occurring after 5 mins at 60°C (Day & Mercer 1964). IIV6 infectivity has been reported to be reduced by solar UV light and ultraviolet radiation, especially in artificial aquatic habitats (Hernandez et al. 2005; Ince et al. 2018). The epizootic haematopoietic necrosis virus (EHNV), an iridovirus of the genus Ranavirus which affects frogs and salamanders, has been reported to be susceptible to heating to 60°C for 15 mins (OIE 2021h), however some ranaviruses remain infectious after desiccation (Chinchar et al. 2021). Red sea bream iridovirus (RSIV), which causes significant mortality in farmed red sea bream (*Pagrus major*) and several other species of farmed marine fish, has been reported to be inactivated at 56°C for 30 mins, sensitive to ether and chloroform, inactivated by formalin (0.1%) and stable in tissue at –80°C (Nakajima et al. 1999; Nakajima & Sorimachi 1994; OIE 2021m).

8.2.2 Epidemiology

Host range

Species which are reported to be susceptible to infection (N= natural; E= experimental exposure) with DIV1 include:

- *Cherax quadricarinatus* [N] (Xu et al. 2016)
- *Exopalaemon carinicauda* [E] (Chen et al. 2019a)
- *Macrobrachium nipponense* [N] (Qiu et al. 2019a)
- *Macrobrachium rosenbergii* [N] (Qiu et al. 2019a)
- *Penaeus vannamei* [N, E] (Qiu et al. 2017; Qiu et al. 2018a)
- *Penaeus japonicus* [N, E] (He et al. 2021b; Qiu et al. 2019a; Qiu et al. 2018c)
- *Penaeus monodon* [N, E] (He et al. 2021a; OIE 2020b; Srisala et al. 2020a)
- *Portunus trituberculatus* [N, E] (crab) (Qiu et al. 2021)
- *Procambarus clarkii* [N, E] (Qiu et al. 2019a; Xu et al. 2016).

DIV1-positive PCR results have been reported in a few species (health status of the animals is not specified in the reports or is unknown) (N= natural; E= experimental exposure), including:

- Antarctic krill [E] (China Fisheries Channel 2020; NACA 2020b)

- *Eriocheir sinensis* [E] (crab) (Pan et al. 2017)
- *Helice tientsinensis* [N] (crab) (Qiu et al. 2021)
- *Hemigrapsus penicillatus* [N] (crab) (Qiu et al. 2021)
- *Macrobrachium superbum* [N] (Qiu et al. 2019a)
- *Nereis succinea* [E] (clam worm) (China Fisheries Channel 2020; NACA 2020b)
- *Pachygrapsus crassipes* [E] (crab) (Pan et al. 2017)
- Polychaetes [N] (Harkell 2020b; NACA 2020a)
- *Penaeus chinensis* [N] (Qiu et al. 2017)
- *Penaeus merguiensis* [E] (Liao et al. 2020).

DIV1-positive PCR results have been reported in a few species, but no clinical signs of infection were found (N= natural exposure)

- *Cladocera* species [N] (water flea) (Chen et al. 2019a; Qiu et al. 2019a)
- *Pomacea canaliculata* [N] (apple snail) (Qiu et al. 2020a)
- *Plexippus paykulli* [N] (jumping spider) (Qiu et al. 2020a).

Cladocera spp., *P. canaliculate* and *P. paykulli* are not considered a DIV1 susceptible species (Chen et al. 2019a; Qiu et al. 2020a), although they may act as a vectors. *Cladocera* spp. showed no histopathological features typical of DIV1 infection or positive signal by *in situ* DIG-labelling- loop-mediated isothermal amplification (ISDL) (Qiu et al. 2019a). DIV1 was not detected in tissues from *P. canaliculate* or *P. paykulli* following TEM or ISDL (Qiu et al. 2020a).

It is reported that wild polychaetes have been found positive for DIV1 (NACA 2020a) and that they carry the virus in the intestinal track (Harkell 2020b) but no further details were provided about these reports.

Frozen Antarctic krill and clam worm (*Nereis succinea*) have been reported to be susceptible to DIV1 following experimental studies, but no details of the testing methods or experimental protocols were given in these publications (China Fisheries Channel 2020; NACA 2020b). *P. merguiensis* challenged by intramuscular injection of DIV1 mounted an immune response 48 hours post-exposure (Liao et al. 2020). It was not investigated if there were any changes consistent with DIV1 infection or associated mortality, but it is noted that the study occurred only over 48 hours. It is therefore unknown if *P. merguiensis* can be considered a susceptible species. Liao et al. (2020) reported that the DIV1 inoculum was obtained from infected *P. merguiensis*, however no further details were provided about the health status or exposure route of the animals from which the DIV1 was sourced. There are no reports of mortalities associated with DIV1 infection in *P. merguiensis* (Liao et al. 2020).

The crab *Portunus trituberculatus* has been confirmed as susceptible to infection with DIV1 following experimental infection by *per os* and intramuscular injection (Qiu et al. 2021). DIV1 has also been reported in other crab species, with infection speculated but not confirmed (Pan et al. 2017; Qiu et al. 2021).

DIV1 has been observed in farmed prawns of all sizes in China (China Fisheries Channel 2020). In other reports it is stated that infection with DIV1 on farms in China occurred in 2–7cm *P. vannamei*

(Qiu et al. 2017; Qiu et al. 2019a), 4–6cm *M. rosenbergii* and 5–7cm *Pr. clarkii* (Qiu et al. 2019a). Symptoms and mortality caused by infection with DIV1 in experimentally infected *P. vannamei* have been observed from postlarvae to sub-adult prawns (Qiu et al. 2017). DIV1 has also been detected by PCR in grossly normal wild adult *P. monodon* of potential broodstock size (Srisala et al. 2020a).

Geographical distribution

DIV1 was first reported (as CQIV) in 2014 from farmed *C. quadricarinatus* in Fujian province, China (Xu et al. 2016). In the same year, DIV1 was reported in a prawn farm in Zhejiang province, China (Qiu et al. 2017). Further PCR surveys in provinces across China showed that DIV1 was present in surrounding prawn farming areas (Chen et al. 2019a; Qiu et al. 2017; Qiu et al. 2019a). Results of the epidemiological survey also suggested that the 2014 outbreak in Zhejiang might not have been the first (Qiu et al. 2017). Early in 2020 an outbreak of DIV1 was reported in the prawn farming province of Guangdong in China (The Fish Site 2020). DIV1 has been detected by PCR in grossly normal *P. monodon* caught from the Indian Ocean (Srisala et al. 2020a). Infection with DIV1 in farmed *C. quadricarinatus*, *P. vannamei* and *P. monodon* has been reported in Taiwan (Cheng 2020; Chung 2020; OIE 2020b; Su-min, Shen & Yi-ching 2020). There have been suspicions of DIV1 detections in other countries, but these have not been officially reported.

Prevalence

Surveys from farmed stocks in provinces of China have reported infection prevalence ranging from 0–25% (Qiu et al. 2017; Qiu et al. 2018a). In China during 2014–2016, 625 farmed prawns were collected from ponds distributed in 7 provinces. Nested PCR showed that 15.8% (99/625) of those samples were DIV1-positive (Qiu et al. 2017). DIV1-positive PCR results per species were 15.5% (89/575) in *P. vannamei*, 15.2% (5/33) in *P. chinensis*, 50% (5/10) in *M. rosenbergii* and 0% (0/7) in *P. japonicus* (Qiu et al. 2017). A later survey of 323 samples collected from *P. vannamei* ponds in Zhejiang province in China found 25.7% (83/323) were positive for DIV1 by qPCR (Qiu et al. 2018a). Surveys for the presence of DIV1 in crab farms in Shandong Province, China detected DIV1 at prevalence of 36% (4/11 samples) and 22% (2/9 samples) in two out of five *Po. trituberculatus* farms (Qiu et al. 2021). Targeted surveillance for DIV1 in China during 2017–19 detected it in 13 of 16 provinces ((Qiu et al. 2021) citing (Qiu et al. 2019c, 2020c; Qiu et al. 2018c)).

DIV1 has been detected by nested PCR in about 19% (5/26) of wild, grossly normal *P. monodon* broodstock caught from the Indian Ocean (Srisala et al. 2020a). In addition, 6.9% of wild *H. tientsinensis* (2/29) and 32.5% of *He. penicillatus* (26/80) collected from the drainage channels of crab and penaeid prawn farms in China tested positive for DIV1 by PCR. No investigation was done to determine if the animals had clinical signs of disease, however the authors speculated that the relatively high viral load of DIV1 detected in the animals indicated they were infected and not just carrying the virus (Qiu et al. 2021).

Mortalities

Mortalities of over 80% in farmed prawns and crayfish have been reported in China due to DIV1 (Qiu et al. 2017; Qiu et al. 2019a; Xu et al. 2016). Mortalities due to DIV1 are usually associated with bad water quality and environmental conditions (Tran 2018; Wright 2019). For example, it has been suggested that an outbreak of DIV1 in China was due to over wintered, pond-reared broodstock which had developed DIV1 and passed it through to the first crop (Harkell 2020b).

Mortalities due to DIV1 appear to vary with prawn species and are reported to be higher in *P. vannamei* than *P. monodon* (Harkell 2022). Taiwan reported mortality rates of 20% in a *P. monodon* farm, and 0%, 20% and 90% in three DIV1 infected *P. vannamei* farms (OIE 2020b). Mortalities in farmed *C. quadricarinatus* (0.13%) were also reported in Taiwan but all animals on the affected farms were destroyed once infection was detected (OIE 2020c).

No reports of mortalities in crabs due to natural infection with DIV1 were found. However, experimental infection of *Po. trituberculatus* with DIV1 resulted in 50% (3/6) and 100% (6/6) mortalities following *per os* and intramuscular injection, respectively (Qiu et al. 2021). In this study, *per os* infected crabs were fed minced DIV1-infected *P. vannamei* tissues at a dose of 5% of total bodyweight after 24 h starvation. Intramuscular injection of crabs was done using a DIV1 inoculum containing 104 copies/µL at a dose of 2 µL/g body weight (Qiu et al. 2021).

Transmission

The natural mode of transmission of DIV1 is unknown. However, experimentally, oral transmission of DIV1 has been achieved (Qiu et al. 2017; Qiu et al. 2018a). Experimentally, DIV1 has also been transmitted by anal reverse gavage and by intramuscular injection (Liao et al. 2020; Pan et al. 2017; Qiu et al. 2017; Xu et al. 2016). An outbreak in Taiwan was speculated to be due to ponds being infected by migratory birds translocating prawns, or through the introduction of imported prawn postlarvae which were contaminated with DIV1 (Chung 2020). DIV1 transmission between ponds and across crustacean species has been reported to be due to a lack of on-farm biosecurity (Qiu et al. 2019a) or using live polychaetes as feed (Harkell 2020b).

There are no reports demonstrating that DIV1, or other crustacean iridoviruses can be transmitted via water; however, most invertebrate iridoviruses are highly stable in water (Ince et al. 2018) and the aquatic iridovirus, RSIV, is transmitted via water (OIE 2019b).

Crabs have been proposed as potential sources for the introduction of DIV1 into prawn aquaculture systems. This was suggested following *per os* challenge of *P. vannamei* (6 cm) with DIV1-infected *P. trituberculatus* that resulted in 100% (4/4) mortalities and PCR positive results in the challenged prawns. No histological studies were done of the prawns in this study (Qiu et al. 2021).

Mechanism of spread

The mechanism of DIV1 spread into new countries and/or areas has not been determined. DIV1 is expected to have horizontal transmission (Qiu et al. 2017; Qiu et al. 2018a). Movement of live animals, particularly broodstock and postlarvae, and international trade of raw prawns may facilitate the introduction of DIV1 into new areas.

Infectious dose

The minimum infectious dose of DIV1 required to cause disease in susceptible species by experimental challenge or natural infection is unknown. *P. vannamei* (6cm) fed once with minced DIV1-infected tissues (5mm^3) at 5% of total body weight became infected with DIV1 (Qiu et al. 2017). Cumulative mortalities reached 100% within 2 weeks of post-infection (Qiu et al. 2017). DIV1 has also been successfully transmitted to *P. vannamei* by 15µl intramuscular injection and 200µl reverse gavage with a 100× dilution of purified crude extracts of DIV1 from infected cephalothoraxes (Qiu et al. 2017). *Per os* bioassays in *E. carinicauda* showed cumulative mortality of 50 ± 26.5% following feeding with 3g DIV1-infected cephalothoraxes of *P. vannamei* with a viral load of about

10^{10} copies/g (Chen et al. 2019a). Intramuscular injection with 2µl/g body weight of a $1:10^6$ dilution of purified DIV1 resulted in lethal infections in *P. vannamei, C. quadricarinatus* and *Pr. clarkii* (Xu et al. 2016). Intramuscular injection of *P. monodon* with 50µl of DIV1 inoculum at six concentrations was used to calculate the LD_{50} and evaluate the virulence of DIV1. Inoculated *P. monodon* showed clinical symptoms and tested positive by PCR for DIV1 when the concentration of DIV1 in the inoculum was $\geq 1.15 \times 10^7$ copies/ng DNA. Also, the death rate of *P. monodon* increased with the increase in viral copies and reached 100% mortality within 10 days. However, when the concentrations of DIV1 was $\leq 1.15 \times 10^6$ copies/ng DNA, the inoculated *P. monodon* showed no obvious symptoms (despite PCR-positive results) and the survival rate was 100% after 15 days (He et al. 2021a). Intramuscular injection of 50µl of a supernatant containing DIV1 at a concentration of 1.5×10^5 copies/µg DNA into *P. merguiensis* resulted in differential expression of genes, including 13 immune-related genes, 48 hours post-exposure (Liao et al. 2020).

8.2.3 Pathogenesis

Variation in disease severity and mortalities due to DIV1 appear to occur across prawn species. Prawn aquaculture practice in the Chinese provinces Guangdong and Hainan was reported to switch from *P. vannamei*.to *P. monodon* due to DIV1 disease outbreaks, and in order to achieve higher survival rates (Harkell 2022). In Guangdong province, survival rates as high as 90% in *P. monodon* were reported in comparison to survival rates of 5% in *P. vannamei* (Harkell 2022).

DIV1 infection can lead to damage of the intestinal mechanical barrier, imbalance of oxidative and antioxidant capacity, decrease of immune enzyme activities, and disturbance of digestive enzyme activities in the prawn (He et al. 2022). These cause secondary bacterial infections, including infections with *Photobacterium* and *Vibrio* species (He et al. 2022).

Tissue tropism

DIV1 is reported to mainly infect the hematopoietic tissue and haemocytes (Qiu et al. 2017; Qiu et al. 2018a; Xu et al. 2016). DIV1 has also been reported in antenna, uropods, pleopods, peripods, gill, muscle, hepatopancreas, lymphoid organ, antennal gland and connective tissue (Qiu et al. 2018a; Qiu et al. 2019a; Srisala et al. 2020a; Xu et al. 2016).

Tissue titre

Quantification of the relative copy number of DIV1 in naturally infected *M. rosenbergii* showed that hematopoietic tissue contained the highest DIV1 load (relative abundance of 25.4 ± 16.9%). Hepatopancreas and muscle contained the lowest DIV1 loads (relative abundance of 2.44 ± 1.24% and 2.44 ± 2.16%, respectively) (Qiu et al. 2019a).

Qiu et al. (2018a) compared the relative copy number of DIV1 by qPCR in *per os* challenged *P. vannamei* (mean length 8cm) tissues and found that the highest copy number of virus was detected in haemolymph (average of 1.37×10^9 DIV1 copies/µg DNA). DIV1 was also present at 2.64×10^8 copies/µg DNA in rostrum, 2.38×10^8 copies/µg DNA in antennal flagellum and 1.53×10^8 copies/µg DNA in uropods. Pleopods, gills, hepatopancreas and muscle presented lower concentrations of DIV1. Muscle contained the lowest concentration of DIV1 copies (average 1.19×10^7 DIV1 copies/µg DNA), which was 110 times lower than that detected in haemolymph (Qiu et al. 2018a).

Intramuscular injection of *P. monodon* with 50 µL of a DIV1 inoculum containing 1.15×10^7 copies/ng DNA resulted in clinical signs of DIV1 and mortalities in the challenged prawns. Quantitative detection of DIV1 in the hemocytes, hepatopancreas, intestine, gills, and muscle at 0, 6, 12, 24, 48, 72, 96 hours post-infection showed that DIV1 load increased with time in all tissues, and that the DIV1 content in hemocytes was the highest at all time points (He et al. 2021a). Also, *P. monodon* exposed to DIV1 by intramuscular injection with 50 µL of inoculum containing 1.15×10^4, 1.15×10^5 or 1.15×10^6 copies/ng DNA showed no clinical signs of disease, but DIV1 was detected by PCR. Quantitative detection of DIV1 in the same tissues showed that DIV1 copy number remained at a level lower than 1×10^4 copies/ng DNA at 15 days post-exposure (He et al. 2021a). DIV1 was present at 1.15×10^{10} copies/ng DNA in the muscle of *P. monodon* that were infected by feeding on DIV1 infected tissue (He et al. 2021a).

8.2.4 Diagnosis

Clinical signs

The clinical signs associated with DIV1 are not specific. In *P. vannamei,* gross signs of DIV1 infection include empty stomach and guts, pale hepatopancreas and soft shell. Slightly reddish body is present in one third of infected prawns (Qiu et al. 2017). Enlarged and deepened pigmentation spots on the shell of the prawn can also be present. Serious cumulative mortalities that occur at the bottom of the ponds, and therefore remained hidden from farmers, have been reported (Qiu et al. 2020a).

Clinical symptoms in experimentally infected *P. monodon* include empty stomach and gut, atrophy and pallor of the hepatopancreas, blackened body and soft shell. Black edge of the abdominal shell was also reported in dead *P. monodon* (He et al. 2021a).

DIV1 infection in farmed *C. quadricarinatus* is associated with lethargy, anorexia, and mortalities (Xu et al. 2016).

In *M. rosenbergii*, clinical signs of DIV1 include empty stomach and guts, pale hepatopancreas and a typical white triangle under the carapace at the base of rostrum (NACA 2020a). Due to this, infection with DIV1 has also been referred to as ‘white head’ or ‘white spot’ in some publications (Qiu et al. 2019a). Slightly whitish muscle and mutilated antenna is also present in some infected *M. rosenbergii* (Qiu et al. 2019a). Experimentally infected prawns and crayfish show cessation of feeding and flaccidity (Xu et al. 2016). Grossly normal DIV1-PCR positive animals have also been reported (Srisala et al. 2020a) however the study did not report if the prawns had histopathological signs of infection or replicating virus.

No obvious clinical signs of DIV1 have been associated with DIV1 infection in crabs (Qiu et al. 2021). Experimentally infected crabs have shown abnormal behaviour, including decreased vitality, retarded reaction, and anorexia (Qiu et al. 2021).

Pathology

Histopathological examination of tissues from DIV1-infected *P. vannamei* revealed basophilic inclusions and pyknosis in hematopoietic tissue and haemocytes in gills, hepatopancreas, periopods and muscle (Qiu et al. 2017; Sanguanrut et al. 2020). Disorganization of the lymphoid organ-tubule matrix accompanied by abnormal morphology of the nuclei and the presence of karyorrhectic and pyknotic nuclei has also been reported in moribund *P. vannamei* from experimental infections (by injection) (Sanguanrut et al. 2020).

Similarly, experimentally infected *P. trituberculatus* showed eosinophilic inclusions and karyopyknosis in hemocytes of gills, hepatopancreas, ovaries, and muscle (Qiu et al. 2021).

Testing

qPCR assays that target the major capsid protein (MCP), the viral ATPase gene, and the putative genes ORF 51R (putative papainase gene), ORF 124R (putative cell surface gene) and ORF 114R (putative D5 family NTPase/ATPase gene) have been developed to detect and quantify DIV1 (Gong et al. 2021; Qiu et al. 2018a; Qiu et al. 2020b; Sellars, Franz & Moser 2022). A real-time isothermal recombinase polymerase amplification assay that targets the MCP gene of DIV1, has been developed for field diagnosis (Chen et al. 2019b). Also, two loop-mediated isothermal amplification (LAMP) methods based on the viral ATPase and the RNA polymerase II genes have been described (Chen et al. 2019a; Gong et al. 2021). An *in situ* hybridization protocol that targets a region of the MCP gene of DIV1 is also publicly available (Qiu et al. 2017).

The WOAH *Infection with DIV1: disease card* provides details of the methods currently available for diagnosis of DIV1 as well as the recommended method for definition of suspect cases, presumptive and confirmatory cases (OIE 2020d).

8.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments.

8.2.6 Control

Control measures for DIV1, such as PCR screening, are primarily aimed at preventing the introduction of the virus into susceptible populations. The use of fresh broodstock in the hatcheries is generally thought to remove the likelihood of DIV1 being present in production ponds (Harkell 2020b). Polychaetes have been reported to carry DIV1 in their intestinal track (Harkell 2020b). It has been suggested that prawn broodstock and hatchery facilities use an alternative to live feed or adopt treatment approaches to decontaminate live feeds prior to use (NACA 2020a). Other general husbandry practices for disease control may include the improvement of sanitary conditions as well as good management of farmed prawns (Tran 2018; Wright 2019).

8.2.7 Impact of the disease

Infection with DIV1 has been reported to cause severe disease and high mortality in farmed prawns and crayfish in China (Li, Xu & Yang 2017; Qiu et al. 2017; Xu et al. 2016). Infection with DIV1 was suggested to have contributed to a decline in the annual output of *P. vannamei* in China from 1.5 million tonnes in 2013 to 1.2 million tonnes in 2018 ((China Fisheries Channel 2020) citing the 2019 China Fishery Statistical Yearbook). However, it has also been reported that due to the absence of widescale PCR testing in China, some farm and hatchery operators may be attributing losses caused by infectious hypodermal and hematopoietic necrosis virus, *Enterocytozoon hepatopenaei* and acute hepatopancreatic necrosis disease to DIV1 (Harkell 2020b). Prawn farmers in Guangdong Province in China, have attributed crop losses of up to 95% and US$14,000 to DIV1 outbreaks (China Fisheries Channel 2020; The Fish Site 2020). There are no reports of mortality associated with DIV1 infection in *P. merguiensis* (Liao et al. 2020) and one report of 20% mortality in farmed *P. monodon* (OIE 2020b). In Taiwan, DIV1 was detected in farmed *C. quadricarinatus*, *P. vannamei* and *P. monodon* (Cheng 2020; Chung 2020; Su-min, Shen & Yi-ching 2020). All animals on the affected farms were destroyed (Cheng 2020; Chung 2020; Su-min, Shen & Yi-ching 2020).

Although DIV1 has been detected in the wild (Srisala et al. 2020a), no reports were found about the impacts of infection with DIV1 on wild crustacean populations.

8.2.8 Current biosecurity measures

DIV1 was not assessed in the Prawn IRA 2009 and therefore there are no specific current biosecurity measures.

8.2.9 Conclusion

DIV1 is present in exporting countries, is not present in Australia and is capable of causing adverse effects in Australia. In Australia, infection with DIV1 is a nationally notifiable disease (AHC 2020). Based on the preceding information, risk assessment for DIV1 is warranted.

Infection with DIV1 is an emerging disease and as such, it is noted that the availability of evidence about the susceptibility of many native Australian crustacean species to infection with DIV1 is limited. It is unknown if these species develop clinical signs or are capable of transmitting DIV1 to other species. With respect to Australia's main farmed prawn species, *P. monodon* has been reported to be susceptible to infection with DIV1. The mortality rate in farmed *P. monodon* with DIV1 infection suggests they may be less susceptible than *P. vannamei.* In the case of *P. merguiensis*, it is unknown if they can be infected through natural exposure, and the severity of disease.

As more information becomes available about DIV1, the department will reconsider the risk assessment to ensure the biosecurity risks are appropriately managed.

8.3 Risk assessment

Based on chapter 4 and the technical information about DIV1 presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of DIV1 meets Australia's ALOP are shown in Appendix F.

8.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for DIV1 were that:

- This risk review is generic and therefore the entry assessment assumes that DIV1 is present in all source countries.
- DIV1 infects various penaeid and caridean prawn species of marketable size that are exported to Australia.
- Prevalence of DIV1 of up to 25% have been reported in farmed prawns (Qiu et al. 2017; Qiu et al. 2018a). There is one report of mortality (20%) in farmed *P. monodon* (OIE 2020a). It is not known if *P. monodon* develop clinical signs of infection.
- There is only one report of DIV1 in the wild where it was detected by PCR in grossly normal wild *P. monodon* caught from the Indian Ocean (Srisala et al. 2020a).
- DIV1 would be present in the whole body of infected prawns.
- The viral load of DIV1 in infected imported prawns is likely to be sufficient to cause infection in susceptible species.

- Post-harvest inspection may detect grossly abnormal prawns that are DIV1-positive and remove them before export. Prawns with mild gross signs or which do not show clinical signs would be unlikely to be detected.
- DIV1 in imported prawns is expected to survive freezing, storage and transport and remain infectious at the time of import.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of DIV1 in imported prawns was estimated to be **high**.

8.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for DIV1 were that:

- DIV1 would be present in the whole body of infected prawns or in associated wastes that may enter the environment of the exposure groups.
- DIV1 would be expected to be present in sufficient loads in imported prawns (or associated wastes) to cause infection in susceptible species if exposed.
- DIV1 in imported prawns (or associated wastes) is likely to persist and remain infectious at the point of exposure.
- Important aquaculture and wild-caught species in Australia, including *C. quadricarinatus*, *M. rosenbergii*, *P. japonicus* and *P. monodon* are reported to be susceptible to infection with DIV1. Other DIV1 susceptible species and potential vectors are widespread in Australian waters including a single crab species. The impact of DIV1 on threatened native Australian species such as the critically endangered *Cherax tenuimanus* is unknown. DIV1 has been detected by PCR in grossly normal wild *P. monodon* and in farmed *P. monodon*. It is unclear if infection with DIV1 in *P. merguiensis* causes disease.
- Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustaceans would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a farm has not implemented standards of entry-level biosecurity for intake water that would exclude DIV1 or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.
- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Species susceptible to DIV1 are likely to be present in research facilities and public aquaria.
- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers

(including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley by recreational fishers and because species susceptible to DIV1 are found in Australian waters.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to DIV1 in imported prawns was estimated to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**High**.

8.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to DIV1 in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**High**.

8.3.4 Consequence assessment

Partial likelihood of establishment and spread

The key points considered relevant when determining the partial likelihood of establishment and spread for DIV1 were that:

- DIV1 can be transmitted horizontally through ingestion of infected tissues. It is unknown if DIV1 can be transmitted via water.
- It is unknown if prawns that survive DIV1 infection can remain infectious.
- It is expected that susceptible species feeding on DIV1-infected prawns would receive an infectious dose.
- DIV1 host species include *C. quadricarinatus* and *P. monodon* which are farmed and are also a target species for fisheries in Australia. Other important wild-caught species found in Australia that are susceptible to infection with DIV1 include *M. rosenbergii* and *P. japonicus*.
- *P. merguiensis* are susceptible to infection with DIV1, but it is unknown if they develop clinical signs or disease.
- Other DIV1 susceptible species (for example *P. trituberculatus*) and potential vectors such as *Cladocera* species, the jumping spider *P. paykulli* and polychaetes, are present in Australia.
- It is unknown how long DIV1 can persist in the environment without a host, whether DIV1 can be transmitted via water and whether crustaceans which have been infected with DIV1 and recovered can transmit the virus.
- The likelihood of DIV1 establishment, following a given quantity of DIV1 entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible

animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.

- If establishment of DIV1 were to occur in the wild, spread to other populations is less likely than for farmed crustaceans because infected wild animals (particularly those clinically affected) are likely to be prey for non-susceptible animals and the densities of susceptible animals are much lower which reduces the opportunities for transmission. However, the likelihood of DIV1 in a wild population spreading to its natural geographic limits is greater than for other hazards with limited host ranges and few non-prawn hosts, for example, infectious myonecrosis virus.
- If DIV1 were to establish in the wild, especially in waters around prawn farms, it may spread to farms if it is transmissible through water. In the absence of effective biosecurity measures, wild infected prawns may be transferred into the farms through the inlet water channels along with vectors such as *Cladocera* species, *P. paykulli* and polychaetes. *C. quadricarinatus* are susceptible to DIV1 and may be capable of entering farms through movement across short distances of land. However, given the physiological and physical requirements of *C. quadricarinatus*, this is considered less likely than for the crab *P. trituberculatus*. It is noted that DIV1 has been reported to affect three crab species. Two of these crab species are not reported in Australia; however, related species are present (for example, *Pachygrapsus minutus, Pachygrapsus plicatus* and *Pachygrapsus laevimanus*).
- If DIV1 were to establish on a farm and it were transmissible through water, DIV1 could spread to neighbouring farms and wild populations through effluent water. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of DIV1 be suspected and response measures initiated. It is unknown if or for how long DIV1 could persist in the water column and remain infectious.
- DIV1 host species are found in Australian waters. The likelihood of DIV1 spread from farms to wild populations or neighbouring farms via escaped prawns is reduced due to the systems in place on farms to prevent discharge of live animals, however DIV1 could spread this way.
- If DIV1 were to establish in hatchery crustaceans, spread to wild crustaceans would be unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.
- Spread of DIV1 from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae as prawn species cultured in Australia are susceptible to infection. It is unclear if *P. monodon* and *C. quadricarinatus* postlarvae would show clinical signs of disease at the time of transfer to the farm.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of DIV1 in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**High**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Low**.

Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of DIV1 were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- Australia's main farmed prawn and crayfish species are susceptible to DIV1. *C. quadricarinatus* which is farmed in Australia is susceptible to infection with DIV1 and infections have been associated with high morbidity and mortality. DIV1 has been detected by PCR in grossly normal wild *P. monodon*, and one report of 20% mortality in farmed *P. monodon* from Taiwan was found. Prawn farmers from China have reported higher survival rates during DIV1 disease outbreaks with *P. monodon,* when compared to survival rates in *P. vannamei*. DIV1 has also been detected by PCR in *P. merguiensis* after experimental infection, but it is unknown if DIV1 causes disease in that species. There are no reports of mortalities associated with DIV1 infection in *P. merguiensis*. The limited information about the mortality rates in *P. monodon* and *P. merguiensis* may be because DIV1 is an emerging disease, there is less of an impact of DIV1 on these species or this information has not been widely reported.
- There are no reports on the impacts of infection with DIV1 in the wild, despite DIV1 being detected by PCR in grossly normal wild *P. monodon*. There are no reports of declines in catch rates or associated mortalities. Based on the available information, DIV1 is not expected to impact wild fisheries in Australia.
- Based on the reports of the impacts in China and Taiwan from DIV1 infection, DIV1 establishment and spread would be expected to have a minor impact at the national level on the life or health of susceptible animals.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- Species susceptible to DIV1 and present in Australia include *C. quadricarinatus*, *M. rosenbergii, P. monodon*, *P. trituberculatus* and potentially *P. merguiensis*.
- There are no reports about serious effects of DIV1 on wild crustacean populations in areas where DIV1 is present. Whilst the environmental effects of DIV1 establishment and spread are unknown, they are expected to be limited given the apparent lack of impact in regions where DIV1 is endemic.
- Non-prawn crustaceans such as *Cladocera* species, *P. paykulli* and polychaetes found in Australia may act as DIV1 vectors as they show no signs of infection.
- The direct impact of DIV1 establishment and spread on the environment is expected to be minor at the local level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- DIV1 is listed as a notifiable disease by the WOAH, is included in the *List of diseases in the Asia-Pacific* and is on Australia's *National list of reportable diseases of aquatic animals*. State and territory governments are expected to report on the agent.
- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating DIV1 from wild crustacean populations is unlikely to be launched.
- If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely.
- If a movement regulated area were put in place for an outbreak of DIV1, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.

- Eradication of DIV1 is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Other industries such as seafood suppliers, commercial wild catch fisheries, other freshwater and marine crustacean industries and the bait industry may be affected due to the host range of DIV1.
- Effects can also occur in all potential DIV1 susceptible species which may be indirectly affected by movement regulated areas.
- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- DIV1 infected prawns may show gross signs which may affect their marketability.
- DIV1 establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Infection with DIV1 is a WOAH-listed disease. Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. DIV1 establishment and spread may result in loss of some crustacean export markets due to importing country biosecurity requirements.
- If DIV1 were to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product.
- The impacts of DIV1 establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- The known host range of DIV1 present in Australia includes *C. quadricarinatus*, *P. trituberculatus*, M*. rosenbergii* and *P. monodon*. DIV1 has also been detected by PCR in *P. merguiensis* after experimental infection, but it is unknown if DIV1 is able to cause disease. There are no reports of mortalities associated with DIV1 infection in *P. merguiensis*.
- It is unknown if *Cherax tenuimanus* is susceptible to infection with DIV1. However, *C. tenuimanus* is listed as critically endangered, and if DIV1 were to cause disease in *C. tenuimanus* it could have a significant impact on the survival of this already endangered species.
- Considering the uncertainty surrounding the susceptibility of *C. tenuimanus* to infection with DIV1, a conservative approach has been adopted when considering the susceptibility of native species, particularly those that are endangered or threatened, and it is assumed they are susceptible.
- The impact of DIV1 establishment and spread on the biodiversity of the environment is considered to be minor at the national level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Freshwater and marine crustaceans that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of DIV1 which may impact on social amenity.
- The social impacts of DIV1 establishment and spread are expected to be minor at the local level.

Table 14 shows the individual impact scores for each criteria (determined using Figure 4) for the establishment and spread of DIV1. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 14 Overall impact of establishment and spread of DIV1 for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Minor	E
	The environment (native animals/plants, and non-living environment)	Local	Minor	B
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	National	Minor	E
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	B

Conclusion
The overall impact of establishment and spread of DIV1 was estimated to be **moderate**.

Determination of likely consequences of the outbreak scenario
The likely consequences of the outbreak scenario for DIV1 in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Low**.

8.3.5 Determination of partial annual risk

The partial annual risk of DIV1 entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Very low**.
- Wild crustaceans—**Low**.

8.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with DIV1 in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **low**.

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

8.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for DIV1 in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 8.4.1, 8.4.2 and 8.4.3 present the factors considered and the conclusions reached.

8.4.1 Head and shell removal

When determining if head and shell removal would reduce the overall risk of DIV1 to meet Australia's ALOP, the key points considered were:

- DIV1 is present throughout the whole prawn body but mainly infects the hematopoietic tissue and haemocytes (Qiu et al. 2017; Qiu et al. 2018a; Xu et al. 2016). Significant viral loads have also been reported in other tissues including rostrum, antennal flagellum, uropods, pleopods, gills, hepatopancreas and muscle (Qiu et al. 2018a).
- Head and shell removal is expected to significantly reduce the viral load of DIV1, however, it is not considered to reduce the likelihood of entry of DIV1. This is because it is expected that sufficient DIV1 would still be present in the tail muscle to infect susceptible species if ingested.
- DIV1 was not detected in deveined, de-headed and de-shelled prawns during monitoring programs undertaken during 2020–22. These programs included batches from countries where DIV1 is endemic. Because DIV1 was not detected in these programs, the entry likelihood for DIV1 in these products is reduced based on the known approach rates of DIV1.
- Ongoing monitoring (see section 17.4.2 Ongoing monitoring of batches of imported uncooked prawns) of imported batches for DIV1 is continuing. This will ensure that any changes to the approach rates of DIV1 (and therefore the entry likelihood of DIV1 in these products) is known and additional biosecurity measures can be applied, as necessary.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Head and shell removal is expected to further reduce the likelihood of deliberate exposure of farmed and hatchery crustaceans because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed in research or public aquaria.
- Whole uncooked prawns are preferred over other forms (including uncooked and shelled) of prawns for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There

remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal, applied was determined to be **very low**.

8.4.2 Cooking

When determining if cooking would reduce the overall risk of DIV1 to meet Australia's ALOP, the key points considered were:

- No reported investigations into the stability of DIV1 to heat treatments were found. In general, Iridoviridae virions are inactivated within 30 mins at temperatures over 55°C (Chinchar et al. 2021). RSIV and EHNV, have been reported to be inactivated at 56°C for 30 mins (OIE 2021m) and 60°C for 15 mins (OIE 2021h), respectively. Conversely, IIV6 from the rice stem borer, is thermolabile and was reported to be completely inactivated after 5 mins at 60°C (Day & Mercer 1964).
- Given the uncertainty regarding the effect of heating on DIV1 infectivity, it is assumed that cooking may reduce, but not completely inactivate DIV1. Some infectious virus may remain. Therefore, cooking to attain a core temperature of at least 65°C is expected to reduce the likelihood of entry, but not completely remove it.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **very low**.

8.4.3 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of DIV1 to meet Australia's ALOP, the key points considered were:

- Value-added products (VAP) are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). VAP were not sampled during the monitoring programs undertaken in 2020–22 and therefore there are no known approach rates for DIV1 in this product.
- Therefore, without knowledge of approach rates, it is considered that head and shell removal and processing into a VAP would not reduce the likelihood of entry of DIV1 in the same manner

as it was reduced for products which have had the head and shell removed (refer section 8.4.1 Head and shell removal). Whilst head and shell removal would be expected to reduce the viral load in the prawn, sufficient DIV1 to cause infection in a susceptible species following exposure is expected to remain. It is also not expected that processing into a VAP will reduce the amount of viable DIV1 in the product.

- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

Conclusion

Based on this information, the overall restricted risk of imported frozen uncooked prawns which have been processed into a value-added product, was determined to be **negligible**.

9 *Enterocytozoon hepatopenaei* risk review

9.1 Background

Enterocytozoon hepatopenaei (EHP) is the aetiological agent of hepatopancreatic microsporidiosis (HPM) (Chayaburakul et al. 2004; Tourtip et al. 2009). Disease caused by EHP has also been referred to as enterosporidiosis (Ma et al. 2019). Based on its unique ultrastructural features, EHP has been classified within the Microsporidia phylum and the family *Enterocytozoonidae* (Tourtip et al. 2009).

Penaeus species are naturally susceptible to infection with EHP (Chayaburakul et al. 2004; Tang et al. 2015; Tourtip et al. 2009). The agent now known as EHP was first reported in *Penaeus monodon* from Thailand in 2004 and has since been detected in many parts of Asia and potentially in Venezuela, although in the latter, the similarity in sequence level to type species of EHP is very low (Chayaburakul et al. 2004; Tang et al. 2017; Thitamadee et al. 2016).

Infection with EHP is not listed as notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c). EHP is on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020) and the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2020c). Australia has a long history of passive surveillance and a strong system in place to detect incursions, EHP is considered exotic to Australia.

9.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of EHP is warranted.

9.2.1 Agent properties

EHP is an obligate, intracellular microsporidian parasite in the family *Enterocytozoonidae* that produces infective (mature) ovoid spores and has numerous life stages (Tourtip et al. 2009). EHP spores are approximately 1 µm in length and contain a single nucleus, 5–6 coils of the polar filament, a posterior vacuole, an anchoring disk attached to the polar filament and a thick electron-dense wall (Tourtip et al. 2009). The spore wall consists of two layers, with the outer layer embedded with proteins that act in host cell recognition and in providing support for the spore wall (Vávra & Lukeš 2013). One such protein is the first spore wall protein of EHP (EhSWP1) that contains three heparin binding motifs and is hypothesised to tether spores to host-cell-surface heparin in the hepatopancreas during infection (Jaroenlak et al. 2018).

Microsporidian spores have thick walls and can remain viable for days to years at 4°C in both fresh and marine water and can survive extreme temperatures, variation in pH, and multiple freeze-thaw cycles (Leiro et al. 2012). EHP spores in faecal pellets or dried prawns were found to remain viable for up to 6 months and retain infectivity for over a year under aqueous conditions (Otta et al. 2016). Purified EHP spores kept at 4°C did not completely inactivate even after 5 days (Aldama-Cano et al. 2018).

Experiments on physical and chemical treatments that inactivate EHP spores isolated from infected *Penaeus vannamei* found that complete inactivation of spores in a tissue free suspension was achieved by exposure to:

- freezing at –20°C for at least 2 hours
- 15 ppm $KMn0_4$ for 15 mins
- 40 ppm of 65% active chlorine for 15 mins
- 10 ppm of 65% active chlorine for 24 hours
- 20% ethanol for 15 mins (Aldama-Cano et al. 2018).

Pattarayingsakul et al (2021) concluded that treatment of water containing EHP spores with 20 ppm chlorine (90% active) for 24 hours eliminates EHP infectivity after showing no infection of *P. vannamei* PL12 following exposure by immersion for 16 days in treated water. In contrast EHP contaminated untreated water produced infections in *P. vannamei* PL12 after 6 days of immersion (Pattarayingsakul et al. 2022).

Munkongwongsiri et al. (2020) reported that EHP spores are highly sensitive to heat inactivation at 75°C for one minute after showing that EHP spore extrusion rate and infectivity were abolished on purified spores incubated at this temperature/time combination. Infectivity of EHP spores was tested by challenge of prawns via oral injection and feeding (Munkongwongsiri et al. 2020). Purified EHP spores that were stored at 33°C showed an approximate 50% and 100% reduction in viability after 24 hours and 5 days, respectively (Aldama-Cano et al. 2018).

EHP is considered sensitive to freezing and not able to survive normal commercial freezing temperatures. The World Organisation for Animal Health (WOAH, in the Control methods stated in their EHP disease card) and the Network of Aquaculture Centres in Asia Pacific (NACA) recommends freezing (-20°C for at least 48 hours) to reduce the risk of EHP transmission via live feeds (Flegel 2015b; NACA 2016; WOAH 2022d).

There are few reports published about the effect of freezing on EHP ability to remain infectious.

EHP spore germination studies by Aldama-Cano et al. (2018) demonstrated that freezing purified spores at −20 °C completely abolished spore extrusion from 2 hours onwards (Aldama-Cano et al. 2018). Mai et al. (2020) showed that storing minced EHP-positive hepatopancreas and inoculum at 4°C or –80°C with or without glycerol inactivated EHP as *P. vannamei* orally challenged using the tissue or inoculum did not develop EHP infection (Mai et al. 2020). The study by Mai et al. (2020) did not investigate the effect of –20°C storage on EHP ability to be infectious. For the study reported in Mai et al. (2020) and other research on EHP, the University of Arizona in the United States had previously imported live EHP-infected *P. vannamei* from Thailand to establish infected EHP prawns in their facility (Aranguren [The University of Arizona] 2021, pers. comm., 11 February), demonstrating difficulty in the transmission of infectious EHP from frozen tissue to live prawns under a more natural exposure scenario. Comparably, some studies on the effect of freezing on the infectivity of other Microsporidia species have shown that freezing microsporidium spores results in loss of viability of the spores and loss in their ability to produce infection (Leiro et al. 2012; Shaw, Kent & Adamson 2000; Whitlock & Johnson 1990). Purified microsporidians of the genus *Spraguea* that parasitises Anglerfish were reported to be inactivated by freezing at −20°C for 48 hours (Leiro et al. 2012).

Spores of the microsporidian *Loma salmonae* that parasitises fish were unable to produce infection following freezing at −20°C for 24-48 hours and for 13 days (Shaw, Kent & Adamson 2000). Freeze-drying of spores of the microsporidian *Nosema locustae*, that parasites a wide range locust and grasshoppers, was reported to completely inhibit germination, however the freezing temperature was not specified in the paper (Whitlock & Johnson 1990).

In contrast, Karthikeyan & Sudhakaran (2019) reported transmission of EHP to *P. vannamei* PL9 after 48 hours immersion in an EHP tissue homogenate stored at −20°C. This is the only study found reporting that EHP in tissue homogenate survives freezing at –20°C, however the freezing time was not specified in the report (Karthikeyan & Sudhakaran 2019). There are reports of two microsporidian species which were not affected by freezing at 24 hours, although other time periods have not been reported so it could not be determined if there was an impact of time (Amigó et al. 1996).

The department commissioned the University of Arizona to conduct independent research investigating the effect of freezing on EHP infectivity. In this work, the ability of the EHP to cause infection was assessed by freezing the hepatopancreas of EHP-affected prawns at –18°C for 24 hours, 7 days and 14 days followed by oral challenge of *P. vannamei*. The results showed that freezing at – 18°C for 24 hours does not completely inactivate EHP, however there was a reduction in EHP ability to cause infection as freezing time increased. The results also showed that EHP did not appear to be able to produce infection following 7- and 14-day freezing treatments. This was demonstrated by results from histopathology, *in situ* hybridisation (ISH) and quantitative PCR that showed that: a.) the 24 hours freezing treatment led to a lesser level of infection when compared to the EHP-infected fresh tissue (positive control), and b.) that EHP was not detected by ISH within any of the histological sections of the challenged prawns on the 7- and 14-day freezing treatments (Aquaculture Pathology Laboratory & Department of Agriculture 2022b).

We note that the detection of EHP in the positive controls and the 24 hour treatment groups, demonstrated that the assays were performing as expected. We also note that all treatments in the study, except the negative control, were found to have histological lesions in the hepatopancreas of the prawns (Aquaculture Pathology Laboratory & Department of Agriculture 2022b). To elucidate the cause of these histological lesions, the team at the University of Arizona recommended a follow up analysis of the samples by ISH, as ISH is a sensitive diagnostic technique that shows the precise localization of a specific segment of nucleic acid, in this case EHP, within a histological section. Also, it was possible to carry out ISH in the samples as the study followed the protocol in Lightner (1996b) for fixation with Davidson's AFA (acetic acid, formaldehyde, alcohol), in which prawns were fixed (injected and then immersed) in Davidson's for 26 hours and then transferred to 50-70% ethyl alcohol for storage (Schofield [The University of Arizona] 2022, pers. comm., 12 May). According to Lightner's protocol, once samples are in 50-70% ethyl alcohol, they can be stored indefinitely (Lightner 1996b). As ISH analysis of the samples from 7- and 14-day freezing treatment did not detected EHP within the histological sections, the lesions seen in the hepatopancreas of prawns in these treatments may be due to other factors or to experimental artefacts. Factors can include inactive spores taken up by the epithelial cells of the hepatopancreas or putative inclusion bodies due to a cellular response to limit the invading microbes.

9.2.2 Epidemiology

Host range
Species which are reported to be susceptible to infection (N= natural exposure) with EHP include:

- *Anax parthenope* [N] (dragonfly) (Kumar Dewangan et al. 2023)
- *Ischnura senegalensis* [N] (common bluetail damselfly) (Kumar Dewangan et al. 2023).
- *Pantala flavescens* [N] (dragonfly) (Kumar Dewangan et al. 2023)
- *Penaeus merguiensis* (Otta et al. 2016)
- *Penaeus monodon* [N] (Tourtip et al. 2009)
- *Penaeus stylirostris* [N] (Tang et al. 2015)
- *Penaeus vannamei* [N] (Tangprasittipap et al. 2013).

EHP, or a similar microsporidian within the so-called '*Enterocytozoon* group Microsporidia' (EGM) (Stentiford, Bass & Williams 2019b) was suspected to also infect *Penaeus japonicus* (Hudson, Hudson & Pyecroft 2001), but there has been no evidence to confirm the species.

Dragonflies *A. parthenope, P. flavescens*, and *I. senegalensis* have been reported as susceptible hosts of EHP. EHP was detected by PCR in samples from these species collected from prawn ponds experiencing an outbreak of EHP. Histopathology of the samples showed EHP spores in nymphs and adult dragonflies while *in situ* hybridisation (ISH) showed a positive signal for EHP infection in dragonfly nymphs (Kumar Dewangan et al. 2023).

EHP-positive PCR results have been reported in the following groups (health status of the animals is not specified in the reports) (N= natural exposure):

- *Artemia,* crabs, oysters and squid [N] (Han, Tang & Kim 2018; Kummari et al. 2018; Tang et al. 2015; Tran 2018).

EHP-positive PCR results have been reported in a few species, but no clinical signs of infection were found (N= natural; E=experimental exposure), including:

- *Mytilopsis leucophaeata* [N, E] (Thai false mussel) (Munkongwongsiri et al. 2022)
- *Marphysa gravelyi* [N, E] (mudworm polychaete) (Krishnan et al. 2021)

EHP has been detected in *M. leucophaeata* and *M. gravelyi* by PCR but EHP-positive specimens did not show histopathological signs of disease (Krishnan et al. 2021; Munkongwongsiri et al. 2022). Both, *M. leucophaeata* and *M. gravelyi* are considered vectors of EHP, as they were shown to be able to carry infectious spores for some period of time after ingestion and to transmit them to naïve prawns (Krishnan et al. 2021; Munkongwongsiri et al. 2022).

EHP affects multiple prawn life stages including broodstock and postlarvae (Karthikeyan & Sudhakaran 2019; Pattarayingsakul et al. 2022; Sritunyalucksana et al. 2015b).

Geographical distribution
The causative agent of HPM was first reported as an unnamed microsporidian in *P. monodon* from Thailand in 2004 (Chayaburakul et al. 2004) that was later named EHP in 2009 (Tourtip et al. 2009). EHP has been detected throughout Asia, including Vietnam (Ha et al. 2010; Tang et al. 2015), Brunei Darussalam (Tang et al. 2015), Malaysia (Sritunyalucksana et al. 2015b), India (Biju et al. 2016;

Rajendran et al. 2016), China (Liu et al. 2016), Indonesia (Tang et al. 2016), Philippines (NACA, OIE-RRAP & FAO 2018), Thailand (Munkongwongsiri et al. 2021; NACA, OIE-RRAP & FAO 2020a), Republic of South Korea (Kim et al. 2021) and Taiwan (NACA, OIE-RRAP & FAO 2019b).

A pathogen described as EHP, but likely a different EGM, has also been detected in South America in Venezuela (Tang et al. 2017). EHP has been also detected in Ecuador and Honduras (Moser et al. 2022). Mortalities in *P. japonicus* associated with a morphologically similar microsporidian to EHP were reported in Australia in 2001 (Hudson, Hudson & Pyecroft 2001). This led to speculation that EHP, or at least another EGM, was present in Australasia (Sritunyalucksana et al. 2015b). However, the taxonomy of the parasite was not confirmed (Hudson, Hudson & Pyecroft 2001). Since the description by Hudson et al. 2001, numerous other crustacean-infecting EGM have been described throughout the world (Stentiford, Bass & Williams 2019b). When information about EGMs is considered in conjunction with Australia's strong passive surveillance system which has no evidence of EHP presence in Australia, the parasite described by Hudson et al. in 2001 is not considered to be EHP.

Prevalence

There have been several reports published on the prevalence of EHP in farmed prawn populations in Asia. In India, studies conducted on *P. monodon* and *P. vannamei* samples collected from multiple farms and districts detected EHP at a prevalence of 20–63% (Giridharan & Uma 2017; Rajendran et al. 2016; Thamizhvanan et al. 2019). Additional studies detected EHP in 34% (53/154), 66% (155/235) and 85% (188/219) of prawn ponds tested across multiple districts in India (Behera et al. 2019; Biju et al. 2016; Singaravel, Gopalakrishnan & Martin 2021). *P. vannamei* cultured in freshwater ponds in India had an EHP prevalence of 1.6% (2/126) in samples screened during a surveillance study from 2018–21 (Suryakodi et al. 2022). The prevalence of EHP in *P. vannamei* collected from 40 ponds in China was 68% (494/726) (Shen et al. 2019). In the same ponds, EHP was detected in grossly normal prawns at a prevalence of 11% (17/160) in greenhouse ponds compared to 72% (165/228) in earthen ponds, suggesting that greenhouse ponds may be associated with a lower risk of EHP infection (Shen et al. 2019). EHP was detected at a prevalence of 51% (327/639) in *P. vannamei* samples collected from four regions in Shandong Province of China in 2019–2020 (Hou et al. 2021). A survey of 196 prawn ponds across 133 farms and 7 provinces in Thailand detected EHP in 61% (119/196) (Sanguanrut et al. 2018).

Active surveillance of prawn farms in Thailand in 2019 detected EHP in 26% of prawn samples (sample numbers were not reported) (Gibson 2019). A study comparing EHP infectivity in *P. vannamei* and *P. monodon* found that out of 235 ponds tested in India, 49% (19/39) of *P. monodon* ponds were EHP-positive compared to 69% (136/196) of *P. vannamei* ponds (Biju et al. 2016). Frozen prawns imported into the Republic of Korea from Vietnam and Indonesia had EHP in 29% (17/58) of the samples tested (Han et al. 2019b).

No reports of the prevalence of EHP in wild prawns were found but other EGM have been described from a range of non-penaeid crustacean and fish taxa (Stentiford, Bass & Williams 2019b).

EHP has been reported from polychaetes in the wild. EHP was detected at a prevalence of 7.6% (25/330) in polychaetes samples collected from April 2016 to May 2018 in coastal states in India. From these, 22% (18/82) of polychaetes collected from sites surrounding or adjacent to prawn farms were positive for EHP compared to 3% (7/248) positive cases in samples collected from non-farming

areas. Also, co-infection of EHP and WSSV was detected in 3.6% (12/330) of the samples (Krishnan et al. 2021).

Mortalities

EHP does not usually cause mortality in infected prawns (Thitamadee et al. 2016). A low mortality rate of 1–2% daily has been seen in naturally infected prawns in Vietnam (Tang et al. 2015).

Transmission

EHP has been experimentally shown to be transmitted by cannibalism (Biju et al. 2016; Santhoshkumar et al. 2017; Tang et al. 2016; Tangprasittipap et al. 2013) and it is expected that horizontal transmission through ingestion of infected tissue occurs in natural infections. Cohabitation studies with infected and healthy prawns indicate that EHP can be transmitted via water, likely from spores released into the water from faeces (Aranguren et al. 2021a; Salachan et al. 2017; Tang et al. 2015; Tang et al. 2016). Pattarayingsakul et al (2021) showed that spores released into the water by EHP-infected prawns, remained infective for at least 5 days and in sufficient loads to cause infection in naïve *P. vannamei* PL12. However, assays using EHP-contaminated water that had been rested for 10 days resulted in no detectable infections in naïve PL12 (Pattarayingsakul et al. 2022). Horizontal transmission of EHP between dragonflies and prawns has been experimentally demonstrated. EHP was transmitted to healthy *P. vannamei* by cohabitation with EHP-infected dragonfly nymphs and following *per os* challenge of the prawns with chopped EHP-infected dragonfly nymphs. Transmission of EHP from prawns to dragonfly nymphs was confirmed by cohabitation of EHP-free dragonfly nymphs with EHP- infected *P. vannamei* (Kumar Dewangan et al. 2023).

Transmission of EHP from broodstock to progeny can occur (Vu-Khac et al. 2018) but is likely due to contamination of eggs or early larval stages with faeces (containing EHP-spores) from the mother. Trans-gonadal transmission of EHP is unlikely, as ISH assays conducted in EHP-infected juvenile and broodstock prawns have shown positive signals for EHP in the hepatopancreas, but not in the ovaries or testes of the same prawns (Chaijarasphong et al. 2020).

P. monodon and *P. vannamei* were found to be infected with EHP via live animal feeds in hatcheries (NACA 2016) and PCR screening showed crabs, polychaetes, *Artemia,* oysters and squids were positive for EHP, suggesting these animals may act as vectors (Han, Tang & Kim 2018; Krishnan et al. 2021; Kummari et al. 2018; Tang et al. 2015; Tran 2018). In an epidemiological study, *Artemia* samples were found to contain EHP DNA at 4.3×10^3–4.8×10^5 copies/mg (Piamsomboon et al. 2019). The polychaete *Marphysa gravelyi* and Thai false mussel *Mytilopsis leucophaeata* may act as vectors for EHP (Krishnan et al. 2021; Munkongwongsiri et al. 2022). Naïve *M. gravelyi* became EHP-positive by PCR after being fed with faecal samples from EHP-infected prawns and subsequent oral challenge of naïve *P. vannamei* with these EHP-positive polychaetes resulted in infection (Krishnan et al. 2021). This confirms the viability and infectivity of EHP spores in the polychaete intestine and horizontal transmission of EHP from polychaetes to prawns when used as live feed. Polychaetes may carry EHP only for a period after ingestion as EHP-positive results by PCR were obtained in the polychaetes only when exposed to the spores continuously (either by feeding or by contact with contaminated sediments) (Krishnan et al. 2021). Cohabitation studies showed that *M. leucophaeata* can accumulate EHP spores after being co-cultured with EHP-infected prawns and that the spores they carry can be infectious to naïve *P. vannamei* (Munkongwongsiri et al. 2022).

Mechanism of spread

EHP was first reported in Thailand and subsequently detected in other South-East Asian countries. The mechanism of EHP spread into new countries and/or areas has not been established. Tangprasittipap et al. (2013) suggested that EHP infections in farmed prawns in Thailand resulted by transmission from one or more local reservoir species, as a) EHP occurred after the ponds were stocked; b) EHP was not present in the specific pathogen free (SPF) *P. vannamei* postlarvae used to stock farm ponds; and c) EHP was discovered in indigenous *P. monodon* before it was found in exotic *P. vannamei* (Chayaburakul et al. 2004; Ha et al. 2010; Tangprasittipap et al. 2013).

A pathogen described as EHP has also been reported from Venezuela. However, nucleotide sequence comparison of β-tubulin and spore wall protein genes of the Venezuelan and some South-East Asian EHP isolates indicated that the strain detected was likely not EHP (but another EGM) and that the pathogen had not been introduced to Venezuela from South-East Asia recently prior to the detection (Tang et al. 2017).

Infectious dose

The minimum infectious dose of EHP required to cause HPM in prawns by experimental challenge or natural infection is not known. *Per os* bioassays showed that EHP has been successfully transmitted to *P. vannamei* by ingestions of EHP-infected hepatopancreas tissue (Biju et al. 2016). However, no information was provided of the amount of tissue that was fed to the prawns in the study.

Per os challenge of *P. vannamei* with fresh EHP-infected hepatopancreas (Ct average value of 23.09 quantified by qPCR) resulted in strong positive PCR results and histological lesions characteristics of EHP in the challenged prawns 21 days post-infection (dpi) (Aquaculture Pathology Laboratory & Department of Agriculture 2022b).

Oral challenge of *P. vannamei* with artificial pelleted feed minced with EHP-affected hepatopancreas tissue (EHP load of 10^6 copies/ng of tissue DNA) resulted in severe necrosis in the hepatopancreatic tubules and epithelial cells by 30 dpi. Homogenates from hepatopancreas and faeces from infected prawns analysed by qPCR showed a maximum EHP load at 15 dpi ($7.9 \times 10^6 \pm 2.9 \times 10^6$) followed by 30 dpi ($2.3 \times 10^6 \pm 7.6 \times 10^5$), 90 dpi ($9.5 \times 10^5 \pm 4.5 \times 10^5$) and 60 dpi ($7.7 \times 10^5 \pm 6.9 \times 10^5$) (Kumar et al. 2022).

9.2.3 Pathogenesis

Microsporidia have a characteristic invasion mechanism that involves the polar tube and spore wall. At the first step of infection, the spore wall proteins are capable of interacting with host cell glycosaminoglycans (Southern et al. 2007). Under suitable conditions, spore germination is activated and the polar tube is rapidly extruded to pierce the host cell membrane (Franzen 2004). The polar tube then serves as a channel to transfer an infectious sporoplasm into the host cell to begin the parasitic, intracellular phase of the life-cycle (Franzen 2004). Microsporidian spores can be triggered to germinate *in vitro* by using a combination of nutrients, alterations in temperature, pH, hyper-osmotic conditions, the presence of anions or cations, or exposure to ultra-violet light or peroxides (Aldama-Cano et al. 2018; Keeling & Fast 2002).

It takes approximately 11–15 days for EHP to establish an experimental infection in the hepatopancreas (Jaroenlak et al. 2018; Salachan et al. 2017; Tang et al. 2016). SPF prawns become EHP-infected within 2 weeks when cohabitated with infected prawns, within 1 week when fed EHP-infected hepatopancreas and within 15 days when exposed to pond soil (Chaweepack et al. 2019).

Under experimental conditions EHP infection in *P. vannamei* can occur at a salinity as low as 2 ppt, however, the prevalence and the severity of the EHP infection is higher at a salinity of 30 ppt (Aranguren et al. 2021a). EHP infection results in an increase of biochemical parameters such as total protein, albumin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase where AST and ALT are indicators of tissue damage (Santhoshkumar et al. 2017). This is consistent with histological findings from EHP-infected prawns that show severe degeneration of hepatopancreatic tubules (Rajendran et al. 2016).

EHP has been found in prawns also exhibiting white faeces syndrome (WFS) (Aranguren et al. 2019; Flegel 2012; Ha et al. 2010; Otta et al. 2016; Rajendran et al. 2016; Tang et al. 2016). Rajendran et al. (2016) observed a high prevalence of EHP (96%; 54/56) in prawns collected from a WFS-infected pond compared to 40% (23/58) prevalence in prawns collected from ponds without WFS. In another study, EHP was only detected in prawns from WFS affected ponds and not from the non-WFS ponds, implicating EHP as either the possible cause of WFS or at the very least associated with WFS (Tang et al. 2016). Later, Aranguren et al. (2019) reported a strong association between WFS and EHP, following studies that showed higher EHP copy numbers in prawns from ponds experiencing WFS (about 1×10^7 copies/µl) and ponds with a history of EHP (about 4×10^4 copies/µl) when compared to ponds where WFS was not present nor any clinical sign of diseases were observed (about 4×10^2 copies/µl). This study also reports higher EHP loads in hepatopancreas and faecal strings of prawns from ponds with WFS (average 4×10^7 copies/µl in hepatopancreas, copy number in faecal string not specified) when compared to the ones from ponds without WFS (1×10^5 copies/µl in hepatopancreas). Moreover the study suggest that prawns with WFS could be potentially more infectious of EHP than prawns without WFS (Aranguren et al. 2019). EHP is reported to play a central role in the development of WFS (Aranguren et al. 2021b; Aranguren et al. 2020c). WFS may be the result of the synergistic effects of EHP, possibly acting as a primary pathogen, and an isolate of *V. parahaemolyticus* or other *Vibrio* species, acting as a secondary pathogen (Aranguren et al. 2021b; Aranguren et al. 2020c). The white faeces were composed, almost completely, of large quantities of EHP spores, gut mucus, remnants of sloughed tissues from the hepatopancreas tubules infected with EHP and rod-shaped bacteria (likely *Vibrio* species) (Aranguren et al. 2021b; Aranguren et al. 2020c; Tang et al. 2016). Contrary to this evidence, other studies have shown that EHP infection is detected in both the presence and absence of WFS and that it is unlikely EHP is associated with WFS (Santhoshkumar et al. 2017; Tangprasittipap et al. 2013).

EHP is often present in prawns concomitantly infected with viruses (for example, white spot syndrome virus, infectious myonecrosis virus and decapod hepanhamaparvovirus 1) and bacterial species (for example, *Vibrio* species), suggesting that either EHP is opportunistic in nature and causes infection by exploiting a weakened immune status of the host or conversely infection with EHP weakens the host to be more susceptible to other prawn pathogens (Jithendran et al. 2021; Lee et al. 2022; Sanguanrut et al. 2018; Shen et al. 2022; Singaravel, Gopalakrishnan & Martin 2021; Thamizhvanan et al. 2019; Tourtip et al. 2009). Indeed, the pathogen presumed to be EHP was first detected in prawns that were co-infected with monodon baculovirus (MBV), hepatopancreatic parvovirus (HPV) and *Vibrio* spp. (Chayaburakul et al. 2004). Further, a laboratory challenge study where EHP-infected prawns and healthy prawns were challenged with *Vibrio parahaemolyticus* strains containing Pir toxins (Vp AHPND) resulted in higher mortalities (44–60%) in the EHP-infected prawns compared to healthy prawns (0–18%), suggesting that EHP-infected prawns have a higher susceptibility to Vp AHPND (Aranguren, Han & Tang 2017). EHP has also been found in farmed

P. vannamei showing symptoms of Abdominal segment deformity disease, a disease of undetermined aetiology (Janakiram et al. 2018).

Tissue tropism

EHP replicates within the cytoplasm of tubule epithelial cells of the hepatopancreas (Tourtip et al. 2009). The damage to the hepatopancreas affects prawn digestive and absorptive functioning resulting in poor growth and immunity (Kumar et al. 2022; Otta et al. 2016). Histology and ISH of EHP-infected prawns usually shows various plasmodia stages and mature spores of EHP in the hepatopancreas and gut (Tang et al. 2015). EHP has also been detected in faeces at loads between 2.5×10^3–6.9×10^7 copies/mg of EHP, which is likely sufficient to cause infection (Piamsomboon et al. 2019). There are only two studies that have reported the detection of EHP in muscle of *P. vannamei* by PCR (Karthikeyan & Sudhakaran 2019; Santhoshkumar et al. 2017). However only one of the studies also reported histopathological evidence of EHP in the muscle (Karthikeyan & Sudhakaran 2019) and the study did not perform ISH to corroborate the findings and rule out histological artefacts or disease caused by organisms other than EHP. It has been reported that infection by members of the *Enterocytozoon* group Microsporidia (EGM) is confined to the gastrointestinal tract and directly associated organs of their hosts, without exception (Stentiford, Bass & Williams 2019a).

Tissue titre

The EHP DNA load in the hepatopancreas of naturally infected frozen *P. vannamei* was quantified by qPCR and ranged from 1.7×10^2–1.8×10^7 copies (Han et al. 2019b). The EHP copy number in the hepatopancreas from naturally infected *P. vannamei* (quantified by qPCR) ranged between 2.5×10^2–1.4×10^8 copies/mg (Piamsomboon et al. 2019). In faeces samples, 2.5×10^3–6.9×10^7 copies/mg were detected (Piamsomboon et al. 2019).

9.2.4 Diagnosis

Clinical signs

There are no distinctive gross signs of infection with EHP. Slow growth is the most common sign of disease in EHP-infected prawns (Chayaburakul et al. 2004; Sritunyalucksana et al. 2015b). Reports from prawn farmers indicate that stunted growth begins at 2–3 months cultivation (Salachan et al. 2017). In a more advanced stage infection, EHP-infected prawns have also been found to display soft shells, thin cuticle, lethargy, reduced feed intake, empty midgut, white muscle and black spots on the eyestalk, in muscle tissue and along the hindgut (Aranguren, Han & Tang 2017; Chaweepack et al. 2019).

Pathology

Histology of hepatopancreatic tubule epithelial cells from EHP-infected prawns show the presence of cytoplasmic, basophilic inclusions containing early and late plasmodia as well as mature spores (Chayaburakul et al. 2004; Tang et al. 2015; Tourtip et al. 2009). Mature spores were also observed free in the tubular lumen together with necrotic, sloughed tubular epithelial cells (Chayaburakul et al. 2004; Tourtip et al. 2009). Infected hepatopancreatic tubular epithelial cells that have been sloughed off degrade within the digestive system, resulting in spores being released with the faeces (Otta et al. 2016). Interstitial haemocytic infiltration of the hepatopancreas, enlargement of haemal sinuses and encapsulation of hepatopancreatic tubules were also observed in some cases (Chayaburakul et al. 2004; Rajendran et al. 2016).

Testing

To screen for EHP, PCR and qPCR are commonly used (Han, Tang & Kim 2018; Hou et al. 2021; Jaroenlak et al. 2016; Liu et al. 2018c; Liu et al. 2016; Piamsomboon et al. 2019; Tang et al. 2015; Tourtip et al. 2009; Wang et al. 2020b). *In situ* hybridisation assays (Tang et al. 2015; Tangprasittipap et al. 2013), loop-mediated isothermal amplification (LAMP), rapid isothermal recombinase polymerase amplification assay (RPA) and RPA-Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas12a fluorescence assay methods for detection of EHP have also been developed (Arunrut et al. 2020; Cai et al. 2018; Kanitchinda et al. 2020; Karthikeyan et al. 2017; Ma et al. 2019; Ma et al. 2021; Suebsing et al. 2013; Zhou et al. 2020). Light microscopy of stained hepatopancreas tissue sections or smears can be used to detect EHP but is reliant on finding the characteristic spores that are very small and sometimes only produced in low numbers (Thitamadee et al. 2016). Staining tissue samples with select fluorescent dyes improves the detection and observation of EHP spores (Wang et al. 2020b; Zhao et al. 2020).

9.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments.

9.2.6 Control

Employing good biosecurity measures in prawn farms plays an important role in controlling the spread of EHP infection (Sritunyalucksana et al. 2015b; Thitamadee et al. 2016). Postlarvae and broodstock should be screened by PCR as EHP-negative before using to stock ponds (NACA 2016). SPF stock should also be screened for EHP since many SPF suppliers use the WOAH list of reportable diseases to determine which pathogenic agents SPF stock should be free of (Thitamadee et al. 2016). As EHP was shown to be transmitted by live feeds (for example, polychaetes), farmers are advised to never use live animals as feed for broodstock or to test them by PCR for EHP before use (Sritunyalucksana et al. 2015b; Tangprasittipap et al. 2013; Thitamadee et al. 2016). Alternatively, freezing live feeds at –20°C for 48 hours may be effective at deactivating EHP spores (Aldama-Cano et al. 2018; Leiro et al. 2012). The use of nets on prawn farms to prevent dragonflies from entering ponds may be useful in preventing transmission and spread of EHP through dragonflies (Kumar Dewangan et al. 2023).

In addition to routine diagnosis and monitoring of prawns stocks and feed for signs of infection, pond management protocols should also be implemented (Sritunyalucksana et al. 2015b). After every harvest, ponds should be disinfected and thoroughly dried (at least 3–4 weeks) to ensure EHP spores and vectors are destroyed before stocking (Otta et al. 2016; Sritunyalucksana et al. 2015b). In addition, cleaning all equipment, filters, reservoirs and pipelines of hatchery facilities with 2.5% sodium hydroxide solution is advocated to prevent EHP (Sritunyalucksana et al. 2015b).

9.2.7 Impacts of the disease

Losses due to EHP infection result from severely retarded growth of affected prawns that lead to unprofitable harvests. The economic losses attributed to EHP infection have been rapidly growing as EHP spreads across Asia and EHP is now considered to be a critical threat to prawn aquaculture (Tang et al. 2015). For example, the economic losses in Thailand due to EHP are estimated to be between US$180–232 million per year (Shinn et al. 2018a; Shinn et al. 2018b). Information from prawn farmers is that EHP-infected *P. vannamei* growth arrests at approximately 12g, capping production at approximately 9 tonnes/ha as opposed to the expected target of 12 tonnes/ha. The decision to

harvest early means that farm gate prices for the smaller size prawns are a third lower at US$3.50/kg instead of US$5.30/kg for 18g prawns and production costs are not covered (Shinn et al. 2016). A questionnaire-based survey conducted during 2018–2019 in India estimated that the national production loss due to EHP infection was 1.80 ± 0.24 tonne/ha/crop and the national level annual financial loss was US$ 567.62 million (Patil & Geetha 2021). Geetha et al (2021) estimated a cost of US$ 813/tonne as the average loss due to EHP infection in Indian prawn farming. This average cost of EHP infection was due to loss of production (75.21%) followed by prevention cost (15.22%), extraordinary cost (5.26%, additional labour required for pond management) and treatment cost (4.30%). Geetha et al (2021) also state that the economic impact of EHP infection could be reduced by harvesting the EHP affected crop at a marketable size (Geetha et al. 2022).

No reports were found about the impact of EHP infection on wild prawn populations, which is expected as there are no reports of EHP in wild prawns.

9.2.8 Current biosecurity measures

EHP was not assessed in the Prawn IRA 2009 and there were no biosecurity measures specific for EHP in place. However, during completion of this risk review, the department identified that the biosecurity measures in place (head and shell removal) did not manage the biosecurity risks associated with EHP. Interim import conditions requiring that all uncooked prawns imported for human consumption be deveined (and have had the head and shell removed (last segment and tail fan excluded)) were implemented on 1 July 2020.

9.2.9 Conclusion

EHP is present in exporting countries, is not present in Australia and is capable of causing adverse effects in Australia. In Australia, infection with EHP is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

9.3 Risk assessment

Based on chapter 4 and the technical information about EHP presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of EHP meets Australia's ALOP are shown in Appendix F.

9.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for EHP were that:

- This risk review is generic and therefore the entry assessment assumes that EHP is present in all source countries.
- EHP infects various penaeid prawn species of marketable size that are exported to Australia.
- Prevalence of EHP can range from about 20–85% in farmed prawns. There are no reports of EHP prevalence in wild prawns.
- EHP would be present primarily in the prawn head and gut. EHP is not expected to be present in the muscle of prawns as infection by *Enterocytozoon* is always confined to the gastrointestinal tract and directly associated organs of their hosts.
- The microsporidian load of EHP in infected imported prawns is likely to be sufficient to cause infection in susceptible species.

- Prawns infected with EHP are unlikely to be detected during post-harvest inspection and grading because stunted growth is generally the only physical sign of infection. In more advanced stage infections there may be soft shells, white muscle and black spots. Those prawns would be expected to be detected and removed before export.
- All the available information regarding the effect of freezing on EHP ability to remain infectious was considered. Freezing at –18°C for 24 hours is considered to reduce the load of viable EHP, but it does not completely inactivate EHP. EHP ability to cause infection is reduced as freezing time increases. The exact point between 24 hours and when complete inactivation occurs is unknown. However, there is evidence to suggest that EHP is not able to produce infection in prawns following freezing at –18°C for 7 days because EHP was not detected by ISH analysis following *per os* challenge of prawns with EHP-infected tissue frozen at this time-temperature combination (Aquaculture Pathology Laboratory & Department of Agriculture 2022b).
- EHP in imported prawns is not expected to survive freezing, transport and storage and is unlikely to be infectious at the time of import. Time is a critical factor in the effect of freezing on EHP infectivity. Currently there is not a requirement for the period which product is frozen before import. Therefore, for the entry assessment, it is assumed that imported prawns have been frozen for a minimum of 24 hours and consequently, there will be a reduction in the viability and infectivity of EHP, but not necessarily complete inactivation. Practically, product is likely to be frozen for months before it enters the retail supply chain within Australia.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of EHP in imported prawns was estimated to be **Moderate**.

9.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for EHP were that:

- EHP would be present in the head and gut of infected prawns or in the associated wastes that may enter the environment of the exposure groups.
- EHP would be expected to be present in sufficient loads in imported prawns (or associated wastes) to cause infection in susceptible species if exposed.
- EHP in imported prawns (or associated wastes) is assumed to persist and remain infectious at the point of exposure.
- The main aquaculture and wild-caught species in Australia, including *P. merguiensis* and *P. monodon* are susceptible to EHP infection.
- Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustaceans would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a farm has not implemented standards of entry-level biosecurity for intake water that would exclude EHP or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.

- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Species susceptible to EHP are likely to be present in research facilities and public aquaria.
- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley by recreational fishers and due to the host range of EHP being abundant in Australian waters.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to EHP in imported prawns was estimated to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

9.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to EHP in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Low**.

9.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)
The key points considered relevant when determining the partial likelihood of establishment and spread for EHP were that:

- EHP can be transmitted horizontally by ingestion of infected tissues and via water, likely from spores released into the water from faeces. Transmission from broodstock to progeny may also occur.
- It is expected that susceptible host animals feeding on EHP-infected prawns would receive an infectious dose.
- EHP spores can remain infectious in the water environment for an extended time, with reports showing EHP spores in faecal pellets or dried prawns remaining viable for up to 6 months and retaining infectivity for over a year under aqueous conditions.
- EHP susceptible species and vectors are present in Australia and include the dragonfly *Pantala flavescens*, crabs, polychaetes, *Artemia*, squid and oysters.

- The main prawn species farmed in Australia are susceptible to EHP infection.
- The likelihood of EHP establishment, following a given quantity of EHP entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.
- If one or more index cases of EHP were to occur, establishment and spread in the directly exposed wild crustaceans is more likely compared to most other hazards. This is because EHP infects prawn species present in Australia and prawns affected by EHP do not usually die, which makes their predation by non-susceptible species less likely when compared to animals suffering acute disease. Additionally, EHP spores can remain infectious in the environment for long periods of time without a host and vectors such as crabs, polychaetes, *Artemia*, squid and oysters are present in the wild. Spread to its natural geographic limits may take longer than hazards with broad host ranges which can assist with rapid transmission, but it will be more likely to establish and spread to its natural geographic limits than hazards which cannot survive without a host and have a narrow host range (for example, infectious myonecrosis virus).
- If EHP established in the wild, especially in waters around prawn farms, it may easily spread to farms due to being transmissible through water. In the absence of effective biosecurity fmeasures, wild infected prawns may be transferred into the farms through the inlet water channels along with vectors such as *Artemia*. EHP vectors, harvested from the local area and therefore infected with EHP, such as polychaetes and squid could be deliberately introduced into the farms as feed. It is not known if there are any species of crustaceans susceptible to infection with EHP that are present in Australia and which may be capable of entering farms through movement across short distances of land. For example, crabs have been reported as being carriers for EHP, however the species was not reported. Dragonflies around prawn farms may also play a role in the spread of EHP. EHP has been reported in dragonfly species collected from prawn farms and horizontal transmission of EHP between dragonflies and prawns occurs.
- Spread of EHP from the wild to hatchery crustaceans could also occur through use of EHP vectors, harvested from areas where EHP was established, such as polychaetes and squid as feed in the hatchery.
- If EHP were to establish on a farm it could spread to neighbouring farms or wild populations through faeces in effluent water. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of EHP be suspected and response measures initiated. However, EHP is effectively transmitted through water, and farms which share a common water source with an infected population may be exposed to EHP.
- Spread from farms to wild populations or to neighbouring farms via escaped prawns is reduced due to the systems in place on farms to prevent discharge of live animals.
- If EHP were to establish in hatchery crustaceans, spread to wild crustaceans is unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.
- Spread of EHP from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae as prawn species cultured in Australia are susceptible to infection with EHP. EHP is less likely to spread this way than hazards which do not have significant clinical signs or high mortality, because postlarvae infected with EHP may show signs of slow growth.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of EHP in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

Determining adverse impacts resulting from the outbreak scenario
The key points considered relevant when determining the adverse impacts resulting from establishment and spread of EHP were that:

Direct effects
The effect on the life or health (including production effects) of susceptible animals

- Australia's main farmed prawn species are susceptible to EHP. There is high morbidity and significantly reduced growth rates associated with infection. EHP does not usually cause mortality in infected prawns.
- EHP infection would not be expected to impact wild fisheries in Australia. There no reports of EHP in wild prawns and no reports of declines in catch rates or associated mortalities.
- Based on the impacts in Asia from EHP infection, EHP establishment and spread in Australia would be expected to cause minor impacts at the national level on the life or health of susceptible animals.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There are no reports about effects of EHP infection on wild prawn populations overseas. Whilst the environmental effects of EHP establishment and spread are unknown, they are expected to be limited given the apparent lack of impact in regions where EHP is endemic.
- EHP has been detected in crabs, polychaetes, *Artemia*, squid and oysters. Whilst these species are found in Australia they are proposed to act as vectors rather than susceptible species, so no effect on them is anticipated.
- The direct impact of EHP establishment and spread on the environment is not expected to be discernible at any level.

Indirect effects
The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with EHP is not listed as a notifiable disease by the WOAH, but is on Australia's *National list of reportable diseases of aquatic animals* and the *List of diseases of the Asia-Pacific*. State and territory governments would be expected to report on the agent.
- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating EHP from wild crustacean populations is unlikely to be launched.
- If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely. Particular attention would need to be given to eliminating the EHP spores from the farm.
- If a movement regulated area were put in place for an outbreak of EHP, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.
- Eradication of EHP is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- An EHP outbreak may affect the crab, oyster and bait industries if movement regulated areas are put in place because crabs, polychaetes, *Artemia* species, oysters and squid are possible vectors of EHP.
- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- Stunted growth rates of EHP infected prawns may affect their marketability.
- EHP establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. EHP establishment and spread may result in loss of some crustacean export markets.
- However, if EHP was to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product.
- The impacts of EHP establishment and spread on international trade are likely to be minor at the local level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- No endangered Australian crustacean species, or closely related species, are susceptible to EHP.
- The impacts of EHP establishment and spread on biodiversity are not expected to be discernible at any level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Prawns that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of EHP which may impact on social amenity.
- The social impacts of EHP establishment and spread are expected to be minor at the local level.

Table 15 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of EHP. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer Determining impacts for detailed methodology).

Table 15 Overall impact of establishment and spread of EHP for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Minor	E
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A

Effects	Criteria	Level	Impact	Score
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	Local	Minor	B
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	A
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	B

Conclusion
The overall impact of establishment and spread of EHP was estimated to be **moderate**.

Determination of likely consequences of the outbreak scenario
The likely consequences of the outbreak scenario for EHP in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

9.3.5 Determination of partial annual risk

The partial annual risk of EHP entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Very low.**
- Hatchery crustaceans—**Very low**.
- Wild crustaceans—**Low**.

9.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with EHP in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **low.**

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

9.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for EHP in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 9.4.1, 9.4.2 and 9.4.3 present the factors considered and the conclusions reached.

9.4.1 Head and shell removal

When determining if head and shell removal would reduce the overall risk of EHP to meet Australia's ALOP, the key points considered were:

- In the unrestricted risk assessment, it was considered that freezing for 24 hours played a significant role in managing biosecurity risks of EHP in imported prawns by reducing the likelihood of entry of infectious EHP. However, freezing did not reduce the entry likelihood sufficiently to achieve Australia's ALOP on its own (refer section 9.3.1 Entry assessment).
- Head and shell removal of frozen prawns is not expected to reduce the likelihood of entry of EHP. This is because EHP is present in significant amounts in the gastrointestinal tract of the prawn. Whilst, head and shell removal would reduce the parasite load in the prawn, sufficient EHP to cause infection in a susceptible species following exposure is expected to remain in the gastrointestinal tract.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Head and shell removal is expected to further reduce the likelihood of this occurring because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed for aquatic animals held in research or public aquaria.
- Whole uncooked prawns are preferred over other forms (including uncooked and shelled) of prawns for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal, applied was determined to be **low**.

Therefore, as the overall restricted risk does not achieve Australia's ALOP, additional biosecurity measures applied in combination with head and shell removal are considered necessary.

9.4.2 Head and shell removal plus deveining

When determining if head and shell removal plus deveining would reduce the overall risk of EHP to meet Australia's ALOP, the key points considered were:

- Removal of the gastrointestinal tract and the head and shell is expected to significantly reduce the EHP-load in the prawns. Therefore, head and shell removal plus deveining reduces the likelihood of entry of EHP.
- The additional step of deveining is not expected to reduce exposure likelihoods for farmed or hatchery crustaceans compared to prawns which have only had the head and shell removed.
- The additional step of deveining is also not expected to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley compared to prawns which have only had the head and shell removed. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal plus deveining. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal plus deveining, applied was determined to be **very low.**

9.4.3 Cooking

When determining if cooking would reduce the overall risk of EHP to meet Australia's ALOP, the key points considered were:

- EHP spores are highly sensitive to heat inactivation at 75°C for one minute (Munkongwongsiri et al. 2020). Purified EHP spores stored at 33°C showed an approximate 50% and 100% reduction in viability after 24 hours and 5 days, respectively (Aldama-Cano et al. 2018).
- Given the data regarding the effect of heating on EHP viability, it is assumed that cooking may reduce, but not completely inactivate EHP. Some infectious EHP may remain. Therefore, cooking to attain a core temperature of at least 65°C is expected to reduce the likelihood of entry, but not completely remove it.
- In the unrestricted risk assessment, it was considered that freezing for 24 hours played a significant role in managing biosecurity risks of EHP in imported prawns by reducing the likelihood of entry of infectious EHP (refer section 9.3.1 Entry assessment). However, freezing on its own did not reduce the entry likelihood sufficiently to achieve Australia's ALOP.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **very low**.

9.4.4 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of EHP to meet Australia's ALOP, the key points considered were:

- Value-added products are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). The likelihood of entry of EHP is expected to be the same as for head and shell removal. This includes a reduction in entry likelihood due to freezing and is because it is not expected that the processing will further reduce the amount of viable EHP in the product more than head and shell removal does.

- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

Conclusion

Based on this information, the overall restricted risk of imported frozen uncooked prawns which have been processed into a value-added product, was determined to be **negligible**.

10 Infectious myonecrosis virus risk review

10.1 Background

Infectious myonecrosis virus (IMNV) is the aetiological agent of infectious myonecrosis (IMN) (Lightner et al. 2004; Poulos et al. 2006). IMNV has been tentatively classified within the virus family *Totiviridae* (Bateman & Stentiford 2017; King et al. 2011; Lightner 2011; Lightner et al. 2004; Nibert 2007; Poulos et al. 2006; Tang et al. 2005).

IMNV is known to only infect a limited number of *Penaeus* species, predominantly *Penaeus vannamei* (OIE 2021g). IMN was first reported in farmed *P. vannamei* populations in north-eastern Brazil in 2002 and initially named idiopathic myonecrosis (Lightner et al. 2004). IMNV was later detected in Indonesia and is present in other Asian countries (NACA & FAO 2015b, a; NACA, OIE-RRAP & FAO 2016; Sahul Hameed et al. 2017; Senapin et al. 2007).

Infection with IMNV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Australia has a long history of passive surveillance and a strong system in place to detect incursions, IMNV is considered exotic to Australia.

10.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of IMNV is warranted.

10.2.1 Agent properties

IMNV is an icosahedral, non-enveloped, double-stranded RNA virus, 40nm in diameter with an 8.2 kilobase genome that is most similar to members of the family *Totiviridae* (Dantas et al. 2015; King et al. 2011; Lightner 2011; Lightner et al. 2004; Loy et al. 2015; Nibert 2007; Poulos et al. 2006; Tang et al. 2005). IMNV is a non-enveloped virus and as such, is considered less susceptible to lipid-disruptive cleaning procedures (for example, detergents and pH modification). This stability outside a host means IMNV is likely to survive passage through the gastrointestinal tracts of vectors such as seabirds (Vanpatten, Nunan & Lightner 2004). IMNV has (anecdotally) also been more difficult to inactivate with standard pond disinfection procedures, such as sun drying and chlorination, that are effective against other prawn viruses (for example, Taura syndrome virus (TSV)) (OIE 2021g).

No studies could be found reporting the specific effect of freezing on IMNV viability. However, IMNV sourced from *P. vannamei* infected prawn tissue maintained at –70°C was successfully used in experimental infection trials (Poulos et al. 2006; Sahul Hameed et al. 2017; Tang et al. 2005; Tang et al. 2007c). IMNV can be inactivated by heating at 60°C for at least 3 mins (OIE 2021g).

10.2.2 Epidemiology

Host range

Species which fulfil the criteria for listing as a species susceptible to infection (N= natural; E= experimental exposure) with IMNV in accordance with chapter 1.5 of the WOAH *Aquatic animal health code* (WOAH Code) (WOAH 2022c) include:

- *Penaeus esculentus* [E] (Gudkovs et al. 2015)
- *Penaeus merguiensis* [E] (Gudkovs et al. 2015)
- *Penaeus vannamei* [N, E] (Lightner 2004; Poulos et al. 2006; Tang et al. 2005; Tang et al. 2007c).

Other host species shown to be susceptible to infection with IMNV include (N= natural; E= experimental exposure):

- *Penaeus monodon* [N, E] (NACA 2018; Srisala et al. 2020a; Tang et al. 2005; Tang et al. 2007c)
- *Penaeus semisulcatus* [N] (Aly et al. 2021)
- *Penaeus stylirostris* [E] (Tang et al. 2005; Tang et al. 2007c).

IMNV-positive RT-PCR results have also been reported in a few species, however no active infection was demonstrated (E= experimental exposure), including:

- *Artemia franciscana* [E] (da Silva et al. 2015)
- *Penaeus subtilis* [E] (Coelho et al. 2009).

IMNV affects multiple prawn life stages including postlarvae, juveniles, sub-adults and adults late in the production cycle (OIE 2021g).

Geographical distribution

IMNV first emerged in farmed *P. vannamei* in north-eastern Brazil in 2002 and was later reported in Indonesia and Malaysia (Lightner et al. 2004; NACA, OIE-RRAP & FAO 2018; Senapin et al. 2007; Tang et al. 2005). IMNV has also been reported from China (NACA & FAO 2015b), the Republic of Korea (NACA & FAO 2015a), Myanmar (NACA, OIE-RRAP & FAO 2016), India (Sahul Hameed et al. 2017) and Egypt (Aly et al. 2021).

Prevalence

In a 2004 study of farmed *P. vannamei* in northern Brazil, 9 out of 11 farms sampled had at least one pond test positive for IMNV (Pinheiro et al. 2007). Two further studies in Brazil on farmed *P. vannamei* exhibiting clinical signs of IMN detected IMNV at a prevalence of 53% (37/70) and 90% (27/30) (Feijó et al. 2013; Teixeira-Lopes et al. 2011). IMNV prevalence in *P. vannamei* samples from multiple farms in Indonesia ranged from 55–70% (Rakasana & Laksmi Sulmartiwi 2013; Senapin et al. 2013; Senapin et al. 2011). In India, prawn samples from 3 out of 4 *P. vannamei* ponds tested IMNV-positive (Sahul Hameed et al. 2017). Also, *P. vannamei* cultured in freshwater ponds in India showed IMNV in 2 out of 13 samples screened during a surveillance study carried out from 2018 to 2021 (Suryakodi et al. 2022). In regions where IMNV is enzootic, prevalence may reach 100% (Andrade et al. 2007). A survey of 120 prawn samples collected from 2 *P. semisulcatus* farms in 2 provinces of Egypt detected IMNV at a prevalence of 37.5% (45/120) (Aly et al. 2021).

There is only one report of IMNV in the wild where it was detected in 7.7% (2/26) of grossly normal wild *P. monodon* broodstock captured off Indonesia (NACA 2018; Srisala et al. 2020a).

Mortalities

Mortalities of 20–60% have been reported from IMNV-infected *P .vannamei* ponds (Sahul Hameed et al. 2017; Tang, Pantoja & Lightner 2005; Tang et al. 2005). IMNV may be associated with high mortalities during the acute onset phase of disease, particularly following a stressful event (for example, cast-netting, sudden changes in water salinity or temperature), but progresses to a chronic disease with low-level persistent mortality (OIE 2021g). Also, mortalities are reported to fluctuate with different strains. Three IMNV strains reported in farmed *P. vannamei* from East Java, Indonesia in 2018, were described to be less pathogenic and caused less than 30% cumulative mortality than previous strains (Mai et al. 2019). In contrast, a new strain of IMNV detected in farmed *P. vannamei* from Brazil between 2016 and 2019 was reported to be more pathogenic and to cause mortalities that progressed more rapidly and resulted in a higher cumulative mortality of up to 80% (Andrade et al. 2022).

Transmission

Horizontal transmission through ingestion of infected tissues has been demonstrated (Coelho et al. 2009; Graf et al. 2004; Gudkovs et al. 2015; Sahul Hameed et al. 2017). Horizontal transmission via water also occurs as IMNV has been transmitted to healthy prawns by cohabitation with infected prawns and by bath exposure to water in which the virus is present (Gudkovs et al. 2015). Detection of IMNV replication in spermatophores, mature ovaries, and eggs (fertilised or not) from naturally and experimentally infected broodstock demonstrates that IMNV can be transmitted from broodstock to progeny (da Silva et al. 2016).

Experimental infections have also been induced by injection of purified virions (Poulos et al. 2006; Sahul Hameed et al. 2017; Tang et al. 2005; Tang et al. 2007c) and injection of infected tissue (Gudkovs et al. 2015).

It has been suggested that *Artemia* spp. may act as a vector for IMNV. *P. vannamei* were confirmed IMNV-positive by qRT-PCR (without clinical signs of disease) after feeding on *Artemia franciscana* exposed to IMNV through bath exposure and a virus-phytoplankton adhesion route (da Silva et al. 2015). An earlier study was unable to conclusively link mortality in *P. vannamei* to IMNV following ingestion of live adult *Artemia* spp. previously fed on IMNV-infected prawn tissue (Graf et al. 2004).

Environmental and physical stressors such as extremes of salinity and temperature, cast net collection and possibly, the feeding of low quality diets have also been associated with IMNV outbreaks in *P. vannamei* (Lightner et al. 2004; Vieira-Girão et al. 2015).

Mechanism of spread

The introduction of IMNV into new areas has primarily been attributed to the movement of live animals, particularly broodstock and postlarvae. It has been speculated that illegal transboundary movement of infected broodstock and postlarvae for aquaculture facilitated the introduction of IMNV from prawn farming areas of Brazil to Indonesia (Prasad et al. 2017; Senapin et al. 2007). It has been suggested that the introduction of IMNV into India was via illegally imported broodstock or postlarvae for use in a commercial hatchery (Sahul Hameed et al. 2017). Detection of IMNV by RT-PCR in grossly normal wild *P. monodon* of potential broodstock size caught from the Indian Ocean has been reported (Srisala et al. 2020a). It has been suggested that because *P. monodon* may be infected with IMNV without showing gross signs of disease, the long presence of IMNV in Indonesia after its introduction (in 2007) may have resulted in transfer of the virus from prawn farms to wild

stocks (Srisala et al. 2020a). Srisala et al. (2020) went on to state that if infectious IMNV is widely present in *P. monodon* in the Indian Ocean, it may be possible that an outbreak of IMNV in a *P. vannamei* farm in Malaysia in 2018, occurred as a result of this transmission pathway (Srisala et al. 2020a). It is believed, the farm had movement of wild *P. monodon* onto its premises at the time, although the department can find no evidence of that in the available literature. Nevertheless, the presence of IMNV in the wild poses a potential biosecurity risk for countries who culture *P. monodon* derived from captured stocks, especially for those which co-culture *P. monodon* with species which are susceptible to clinical disease from IMNV, such as *P. vannamei*. Also, re-introduction of IMNV from Indonesia to Brazil was speculated following report of a new IMNV strain in Brazil that was shown to be closely related to IMNV strains from Indonesia based on full-length genome phylogeny (Andrade et al. 2022). This new strain was associated with increased mortalities in Brazilian farms of *P. vannamei* between 2016 to 2021, and farms experiencing mortalities were believed to be raising some unofficially introduced prawn genetic lines from Indonesia (Andrade et al. 2022).

Infectious dose

The minimum infectious dose of IMNV required to cause IMN in prawns by experimental challenge or natural infection is not known. A challenge study did show that injection of healthy *P. vannamei* with IMNV-infected tissue homogenate (~1.0×10^6 IMNV viral copies) resulted in 100% mortality at 52 days post-infection with all prawns testing positive for IMNV by qRT-PCR (Andrade et al. 2007).

Per os bioassays showed that IMNV has been successfully transmitted to *P. subtilis* (weighing 2-3g) by being fed once a day for 3 days with 3.5% bodyweight of infected tissue (Coelho et al. 2009). In other trials, *P. vannamei* (weighing 12–15g) has also been infected by being fed three times with 5% bodyweight infected skeletal muscle (Sahul Hameed et al. 2017)

10.2.3 Pathogenesis

There are studies showing that IMNV can appear as co-infections with *Macrobrachium rosenbergii* nodavirus, white spot syndrome virus (WSSV), *Enterocytozoon hepatopenaei* and infectious hypodermal and haematopoietic necrosis virus (Andrade et al. 2022; Feijó et al. 2013; Jithendran et al. 2021; Senapin et al. 2013; Teixeira-Lopes et al. 2011).

Tissue tropism

IMNV infects striated muscles (skeletal and less often cardiac), connective tissues, haemocytes, lymphoid organ, hindgut, gills and phagocytic cells of the hepatopancreas and heart (OIE 2021g; Tang et al. 2005; Tang et al. 2007c). Skeletal muscle is the primary target tissue for IMNV, this being proposed as a factor relating to the reduced mortality seen with IMNV when compared to infections with TSV, WSSV and the yellow head virus complex, which attack more vital organs of prawns and cause higher mortality within a shorter period (Tang, Pantoja & Lightner 2005; Tang et al. 2005; Tang et al. 2007c).

Tissue titre

Healthy *P. vannamei* injected with IMNV-infected tissue homogenate (~1.0×10^6 IMNV viral copies) resulted in a viral load in the abdominal tissue that ranged from 45–2.27×10^8 copies/µl RNA (Andrade et al. 2007). In another study, naturally infected *P. vannamei* were quantified by qRT-PCR and the IMNV load ranged from 1.26×10^3–5.10×10^5 copies/µg in the pleopods, 3.90×10^3–8.15×10^6 copies/µg in gills, 3.09×10^4–6.85×10^8 copies/µg in muscle and 1.33×10^6–5.08×10^8 copies/µg of total RNA in the haemolymph (da Silva, Pinheiro & Coimbra 2011).

10.2.4 Diagnosis

Clinical signs

IMNV-infected prawns show focal to extensive areas of muscle necrosis, particularly around the distal abdominal segments and tail fan (Lightner et al. 2004). Affected muscles typically have whitish opaque lesions, although white opaque lesions in muscle fibres can be due to other disease agents including non-viral causes (Melena et al. 2012; Senapin et al. 2012; Tang et al. 2005; Yan et al. 2014; Zhang et al. 2014). In some affected prawns, the tail fan may be necrotic and reddened, taking on a cooked appearance (Lightner et al. 2004). Significant hypertrophy of the paired lymphoid organs, an increase of two to four times their normal size, is also a common gross sign (Lightner 2011). As the disease progresses, infected animals become lethargic and may eventually die (Tang et al. 2005). The onset of clinical signs occurs anywhere from 6–13 days following exposure to IMNV in experimentally infected animals (Tang et al. 2005). Healthy, chronically infected animals have also been reported (Lightner et al. 2004; Srisala et al. 2020a; Tang et al. 2005).

Pathology

Histopathology of IMNV-infected prawns show myonecrosis with coagulative necrosis of skeletal muscle fibres, often with marked oedema, in early stages of infection that progresses to liquefactive necrosis with accompanying haemocytic infiltration and fibrosis (Lightner et al. 2004). Perinuclear basophilic inclusion bodies can sometimes be detected in muscle, connective tissue, lymphoid organ and haemocytes (Lightner 2011; Tang et al. 2005). Significant hypertrophy of the lymphoid organ due to spheroid formation is also seen in acutely and chronically infected prawns (OIE 2021g). Many ectopic lymphoid organ spheroids are found in locations other than the main body of the lymphoid organ such as the haemocoelom in the gills, heart, near the antennal gland tubules and ventral nerve cord (Lightner 2011).

Testing

Chapter 2.2.5 of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH Manual) provides details of the methods currently available for targeted surveillance and diagnosis of IMNV (WOAH 2022g). The nested RT-PCR and qRT-PCR methods described in the WOAH Manual are the recommended methods for targeted surveillance to declare freedom from IMNV (Andrade et al. 2007; Poulos & Lightner 2006).

The WOAH Manual also describes the circumstances in which histopathology may be used to obtain a presumptive diagnosis of IMNV infection (WOAH 2022g).

10.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments (OIE 2021g).

10.2.6 Control

Screening broodstock for IMNV using qRT-PCR and discarding prawns that test IMNV-positive has successfully been applied to prevent IMN (OIE 2021g). The development of specific pathogen free prawn stock has proven to be effective for preventing and controlling other viral diseases of prawns and should be applicable to IMNV (OIE 2021g). Selecting for IMNV-resistant lines represents a viable option where IMNV is enzootic (White-Noble et al. 2010). Disinfection of eggs and larvae is recommended to reduce the transmission of IMNV between broodstock and progeny (OIE 2021g).

Environmental stressors such as fluctuations in temperature and salinity have been associated with susceptibility to IMNV outbreaks where the virus is enzootic (Vieira-Girão et al. 2015) and should be avoided or managed where possible. There have been several reports on using RNA interference (RNAi) experimentally as a method to control IMNV infection (Bartholomay et al. 2012; Feijó et al. 2015; Loy et al. 2013; Loy et al. 2012). RNAi inhibits expression of a targeted gene, usually a gene essential for virus replication, resulting in suppression of virus infection and pathology. However, there is no evidence that RNAi has been applied to prawn aquaculture facilities. There are reports of a formulation being provided to prawns in Indonesia which has improved IMNV survival rates. The formulation is reportedly made of natural herbal extracts which have an immunostimulatory effect (Rosenberry 2014; Thitamadee et al. 2016). There is little information available aside from the initial report.

10.2.7 Impact of the disease

Losses from IMNV infection result from both mortality and reduced growth (Lightner et al. 2004). Production losses due to IMN were estimated at US$100–200 million in Brazil (Lightner 2011), US$100–200 million in the Americas (Lightner et al. 2012b) and US$1 billion in Indonesia (Lightner et al. 2012b). Annual prawn losses in Indonesia were estimated in 2016 at US$95.6 million (Shinn et al. 2018a).

Although IMNV has been detected in the wild (NACA 2018; Srisala et al. 2020a), no reports were found about the impact of infection with IMNV on wild prawn populations.

10.2.8 Current biosecurity measures

Currently no specific biosecurity measures exist for IMNV in imported prawns or prawn products. The Prawn IRA 2009 determined the unrestricted risk associated with IMNV to be negligible (primarily because at the time *P. monodon* was not known to be naturally susceptible to infection with IMNV) and therefore biosecurity measures were not necessary (Biosecurity Australia 2009).

10.2.9 Conclusion

IMNV is present in exporting countries, is not present in Australia and is capable of causing adverse effects in Australia. In Australia, infection with IMNV is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

10.3 Risk assessment

Based on chapter 4 and the technical information about IMNV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of IMNV meets Australia's ALOP are shown in Appendix F.

10.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for IMNV were that:

- This risk review is generic and therefore the entry assessment assumes that IMNV is present in all source countries.
- IMNV infects various *Penaeus* species of marketable size that are exported to Australia.

- Prevalence of IMNV can range from 37–90% in farmed prawns (Feijó et al. 2013; Senapin et al. 2007; Senapin et al. 2013; Teixeira-Lopes et al. 2011). There is only one report of IMNV in the wild where it was detected in wild *P. monodon* broodstock captured off Indonesia (NACA 2018).
- IMNV would be present in the whole body of infected prawns.
- The viral load of IMNV in infected imported prawns is likely to be sufficient to cause infection in susceptible species.
- Post-harvest inspection may detect grossly abnormal prawns that are IMNV-positive and remove them before export. Prawns with mild gross signs or which do not show clinical signs would be unlikely to be detected.
- IMNV in imported prawns would be expected to survive freezing, storage and transport and remain infectious at the time of import.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of IMNV in imported prawns was estimated to be **high**.

10.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for IMNV were that:

- IMNV would be present in the whole body of infected prawns or in associated wastes that may enter the environment of the exposure groups.
- IMNV would be expected to be present in sufficient loads in imported prawns (or associated wastes) to cause infection in a susceptible species if exposed.
- IMNV in imported prawns (or associated wastes) is likely to persist and remain infectious at the point of exposure.
- The main aquaculture and wild-caught species in Australia, such as *P. monodon* and *P. merguiensis* are susceptible to IMNV infection. *P. monodon*, however, does not develop clinical disease (NACA 2018; Srisala et al. 2020a; Tang et al. 2005; Tang et al. 2007c). Other IMNV susceptible species and vectors are found in Australia.
- Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustaceans would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a farm has not implemented standards of entry-level biosecurity for intake water that would exclude IMNV or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.
- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Species susceptible to IMNV may be present in research facilities and public aquaria.

- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley by recreational fishers. The host range for IMNV is narrow compared to hazards such as WSSV, but its susceptible species are widespread in Australian waters, and are likely to encounter imported prawns used as bait or berley.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to IMNV in imported prawns was estimated to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

10.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to IMNV in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

10.3.4 Consequence assessment

Partial likelihood of establishment and spread
The key points considered relevant when determining the partial likelihood of establishment and spread for IMNV were that:

- IMNV can be transmitted via ingestion of infected tissues, water and between broodstock and progeny.
- Prawns that survive IMNV infection can remain infectious and become sources of the virus.
- It is expected that susceptible host animals feeding on IMNV-infected prawns would receive an infectious dose.
- The main prawn species farmed in Australia, *P. monodon* and *P. merguiensis* are susceptible to IMNV infection. Only infected *P. merguiensis* show clinical signs. *P. monodon* seem to be refractory to clinical disease.
- Other IMNV susceptible host species are found in Australian waters and include species important for the wild-caught fishery industry, such as *P. esculentus*.
- The likelihood of IMNV establishment, following a given quantity of IMNV entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.

- If establishment of IMNV were to occur in the wild, spread to other populations is less likely than for farmed or hatchery crustaceans because infected wild animals (particularly those clinically affected) are likely to be preyed upon by non-susceptible animals. The densities of susceptible and infected animals are much lower which reduces the likelihood of transmission. However, as *P. merguiensis* and *P. esculentus* are the only host species present in Australia which shows clinical signs due to IMNV infection, the potential for infected wild crustaceans to be preyed upon is less than for other hazards where significant clinical signs are seen. In the case of IMNV the number of susceptible species in Australia is very limited and therefore the likelihood of spread from the wild to its natural geographic limits is less than for hazards such as WSSV.
- If IMNV were to establish in the wild, especially in waters around prawn farms, it may spread to farms due to being transmissible through water. In the absence of effective biosecurity measures, wild infected prawns may be transferred into the farms through the inlet water channels along with vectors such as *Artemia*. There are no known crustacean species susceptible to infection with IMNV that are capable of surviving and moving outside of the water column, for example crabs. Therefore, infected wild crustaceans would only be able to enter farms through the water inlet channels, and not via movement across land.
- If IMNV were to establish on a farm it could spread to neighbouring farms and wild populations through effluent water. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of IMNV be suspected and response measures initiated. However, IMNV is effectively transmitted through water, and susceptible animals which share a common water source with an infected population may be exposed to IMNV. Although it is unknown how long IMNV can persist in the water column and remain infectious. Because *P. monodon* do not show clinical signs of disease, there is an increased likelihood for spread of IMNV from a farm because it may not be obvious that IMNV is present.
- Spread from farms to wild populations or neighbouring farms via escaped prawns is possible, although likelihood is reduced due to the systems in place on farms to prevent discharge of live animals.
- If IMNV were to establish in hatchery crustaceans, spread to wild crustaceans would be unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.
- Spread of IMNV from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae. IMNV is more likely to spread this way than hazards which have significant clinical signs or high mortality as *P. monodon* broodstock and postlarvae will not be identified as diseased. *P. merguiensis* broodstock may show clinical signs if actively infected, but if they have been infected and recovered, they could still pass IMNV to the progeny without showing clinical signs. Postlarvae do not usually show signs of disease until after transfer to the farm.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of IMNV in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Very low**.

Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of IMNV were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- No mortalities have been reported in IMNV-infected *P. monodon, P. merguiensis* or *P. esculentus*. Only infected *P. merguiensis* show clinical signs; *P. monodon* seem to be refractory to clinical disease.
- IMNV is not expected to impact wild fisheries in Australia. There have been no reports of declines in catch rates or associated mortalities in wild fisheries due to IMNV infection.
- IMNV would be expected to have a minor impact at the district or region level on the life or health of susceptible animals.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There is no evidence that IMNV causes serious disease in non-penaeid species or freshwater crustaceans.
- Based on the absence of serious effects of wild prawn populations overseas, the environmental effects of IMNV establishment and spread are expected to be limited.
- The direct impacts of IMNV establishment and spread on the environment is not expected to be discernible at any level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with IMNV is listed as a notifiable disease by the WOAH and is included on Australia's *National list of reportable diseases of aquatic animals.* State and territory governments would be expected to report on the agent.
- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating IMNV from wild crustacean populations is unlikely to be launched.
- If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely.
- If a movement regulated area were put in place for an outbreak of IMNV, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.
- Eradication of IMNV is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- *P. merguiensis* infected with IMNV are likely to show gross signs which may affect their marketability.

- IMNV establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- IMNV is a WOAH-listed disease. Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases, including IMNV. IMNV establishment and spread may result in loss of some crustacean export markets due to importing country biosecurity requirements.
- If IMNV were to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product.
- The impacts of IMNV establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- No endangered Australian crustacean species, or closely related species, are susceptible to IMNV.
- The impacts of IMNV establishment and spread on biodiversity are not expected to be discernible at any level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Prawns that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of IMNV which may impact on social amenity.
- The social impacts of IMNV establishment and spread are expected to be minor at the local level.

Table 16 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of IMNV. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 16 Overall impact of establishment and spread of IMNV for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	District or region	Minor	C
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	A
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	B

Conclusion
The overall impact of establishment and spread of IMNV was estimated to be **moderate**.

Determination of likely consequences of the outbreak scenario
The likely consequences of the outbreak scenario for IMNV in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Very low**.

10.3.5 Determination of partial annual risk

The partial annual risk of IMNV entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Very low**.
- Wild crustaceans—**Very low**.

10.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with IMNV in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **low**.

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

10.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for IMNV in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 10.4.1, 10.4.2 and 10.4.3 present the factors considered and the conclusions reached.

10.4.1 Head and shell removal

When determining if head and shell removal would reduce the overall risk of IMNV to meet Australia's ALOP, the key points considered were:

- Head and shell removal is not expected to reduce the likelihood of entry of IMNV. This is because whilst head and shell removal would reduce the viral load in the prawn, sufficient IMNV to cause infection in a susceptible species following exposure is expected to remain.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Head and shell removal is expected to further reduce the likelihood of this occurring

because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed for aquatic animals held in research or public aquaria.

- Whole uncooked prawns are preferred over other forms (including uncooked and shelled) of prawns for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal, applied was determined to be **negligible**.

10.4.2 Cooking

When determining if cooking would reduce the overall risk of IMNV to meet Australia's ALOP, the key points considered were:

- Crustacean products that have been subjected to heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes (or any time/temperature equivalent that has been demonstrated to inactivate IMNV) are considered safe according to the WOAH aquatic code (WOAH 2022f).
- Given the length of time required to inactivate IMNV in a prawn cooked to a core temperature of 65°C is well outside the time what would be expected for commercial cooking of prawns, it is assumed that cooking may reduce, but not completely inactivate IMNV in imported prawn tissue. Sufficient viable virus to cause disease will likely still be present. Therefore, cooking to a core temperature of 65°C is not expected to reduce the likelihood of entry.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **negligible**.

10.4.3 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of IMNV to meet Australia's ALOP, the key points considered were:

- Value-added products (VAP) are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). The likelihood of entry of IMNV is expected to be the same as for head and shell removal. This is because it is not expected that the processing will further reduce the amount of viable IMNV in the product more than head and shell removal does.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

Conclusion

Based on this information, the overall restricted risk of imported frozen uncooked prawns which have been processed into a value-added product, was determined to be **negligible**.

11 Laem-Singh virus risk review

11.1 Background

Monodon Slow Growth Syndrome (MSGS) was first reported in *Penaeus monodon* in Thailand in 2001 and was named due to the unusual retarded growth that was observed in the prawns (Chayaburakul et al. 2004; Sritunyalucksana et al. 2006a).

It has been shown that Laem-Singh virus (LSNV) is a necessary but insufficient cause of MSGS (Flegel 2012; Pratoomthai et al. 2008; Sritunyalucksana et al. 2006a). LSNV is most closely related to other known RNA viruses in the family *Luteoviridae* (Sritunyalucksana et al. 2006a). LSNV and Wenzhou shrimp virus genotype 9 (WZSV9) have been reported as different isolates of the same virus species (Taengchaiyaphum et al. 2020). Other pathogens, including a small virus-like particle named integrase containing element (ICE) and/or environmental factors may also be involved with LSNV to cause MSGS (Panphut et al. 2011; Sritunyalucksana et al. 2006a).

Various crustacean species are susceptible to infection with LSNV (Chayaburakul et al. 2004; Kumar et al. 2011; Sritunyalucksana et al. 2006a), but only *P. monodon* has been affected by MSGS (Chayaburakul et al. 2004; Sittidilokratna et al. 2009b). MSGS and LSNV have been detected in prawn growing regions of Asia and East Africa (Anantasomboon et al. 2006; Panphut et al. 2011; Prakasha et al. 2007; Sittidilokratna et al. 2009b; Sritunyalucksana et al. 2006a).

Infection with LSNV is not listed as a disease notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c) and is not on the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2020c). MSGS is present on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Australia has a long history of passive surveillance and a strong system in place to detect incursions, LSNV (and WZSV9) are considered exotic to Australia.

Because LSNV is known to be a necessary but insufficient cause of MSGS and it has not been determined exactly the role that LSNV and ICE play in development of MSGS, the following risk assessment takes a conservative approach and considers that infection with LSNV causes MSGS. For the purpose of simplifying this complex situation, within this chapter the cause of MSGS will be referred to as LSNV, even when the literature being cited may not have had that information at the time it was published.

11.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of LSNV is warranted.

11.2.1 Agent properties

MSGS was first noticed in 2001 in Thailand when farmers reported an unusual, abnormally slow growth and a large size variation of *P. monodon*. Initially the cause of MSGS was unknown but a filterable infectious agent was considered to be involved (Anantasomboon et al. 2005; Chayaburakul et al. 2004). LSNV was later proposed to be that filterable infectious agent and a necessary but insufficient cause of MSGS (Flegel 2012; Pratoomthai et al. 2008; Sritunyalucksana et al. 2006a). This conclusion was made when LSNV was detected by PCR and found in association with retinopathy in stunted prawns from MSGS-affected ponds (Flegel 2012; Pratoomthai et al. 2008; Sritunyalucksana

et al. 2006a). LSNV, however, was also detected by PCR in large prawns from MSGS-affected ponds and in prawns from normal (non-MSGS) ponds, in both instances the LSNV was not found in association with retinopathy (Flegel 2012; Pratoomthai et al. 2008; Sritunyalucksana et al. 2006a). These results suggest that for MSGS to occur, presence of LSNV alone, is not enough to cause MSGS, but the LSNV must also be associated with retinopathy. Later, another agent called ICE was found in prawns from MSGS-affected ponds and in absolute association with LSNV in growth retarded prawns (Panphut et al. 2011). The interaction between LSNV and ICE and how this association may cause retarded growth are still unknown (Thitamadee et al. 2016). A second RNA viral-particle of approximately 15nm was observed in MSGS-affected prawns and may be ICE but this was not confirmed (Panphut et al. 2011). The other factors that lead to MSGS are also unknown but may involve other pathogens and/or environmental factors (Flegel 2008, 2009; Rai et al. 2009).

LSNV is an icosahedral, non-enveloped, single-stranded, positive-sense RNA virus 25–27nm in diameter (Anantasomboon et al. 2005; Sritunyalucksana et al. 2006a). LSNV shows amino acid sequence similarity to RNA dependent RNA polymerases of the insect-transmitted plant viruses in the former family *Luteoviridae* and an unassigned *Sobemovirus* (Sritunyalucksana et al. 2006a). It has been reported that LSNV and WZSV9 share 99% sequence identity and are now considered to be different isolates of the same virus species (Taengchaiyaphum et al. 2020). Phylogenetic analysis suggests the most closely related viruses to this species are assigned to the *Sobemovirus*-like group, supporting earlier conclusions on the phylogenetic relationship of LSNV to other viruses (Sritunyalucksana et al. 2006a; Taengchaiyaphum et al. 2020).

There is little information on the stability of LSNV. LSNV infectivity is retained after freezing at −80°C. Inoculum prepared from lymphoid tissues, gill and pleopod of LSNV-infected prawns stored at −80°C and then injected into healthy prawns resulted in LSNV infection (Kumar et al. 2011; Ongvarrasopone, Chomchay & Panyim 2010).

11.2.2 Epidemiology

Host range

The only species in which LSNV has been detected and that has also met the case definition of MSGS (N= natural exposure) is:

- *P. monodon* [N] (Chayaburakul et al. 2004; Flegel 2008; Sittidilokratna et al. 2009b).

Species which were co-cultured with MSGS affected *P. monodon* (E= experimental exposure) and were observed to contain 25nm virus-like particles (TEM, no molecular studies were done), but it was not confirmed that they met the case definition of MSGS, include:

- *Macrobrachium rosenbergii* [E] (Anantasomboon et al. 2008a)
- *Penaeus indicus* [E] (Anantasomboon et al. 2008a)
- *Penaeus vannamei* [E] (Anantasomboon et al. 2005; Anantasomboon et al. 2008a).

Species for which LSNV or ICE (N= natural; E= experimental exposure) have been detected by PCR (infection was not confirmed) include:

- *Metapenaeus dobsoni* [N] (Kumar et al. 2011)
- *Penaeus merguiensis* [N] (Kumar et al. 2011)

- *Penaeus monodon* [N, E] (Kumar et al. 2011; Panphut et al. 2011; Sritunyalucksana et al. 2006a)
- *Penaeus semisulcatus* [N] (Megahed 2019)
- *Penaeus vannamei* [N, E] (Kumar et al. 2011; Sakaew et al. 2008)
- *Scylla serrata* [E] (crab) (Kumar et al. 2011).

LSNV has been detected in multiple prawn life stages, including nauplii, postlarvae, juveniles and broodstock (Kumar et al. 2011; Sakaew et al. 2008; Sittidilokratna et al. 2009b).

Geographical distribution

The first report of MSGS was from farmed *P. monodon* from Thailand in 2001 (Chayaburakul et al. 2004; Sritunyalucksana et al. 2006a). In East Africa in 2004 *P. monodon* were found to meet the case definition of MSGS (Anantasomboon et al. 2006).

LSNV has been identified in both slow-growing and healthy prawn ponds from Thailand, Malaysia, Indonesia, Vietnam, India, Sri Lanka, the Philippines and Egypt (Cruz et al. 2015; Megahed 2019; NACA & FAO 2011; Prakasha et al. 2007; Sittidilokratna et al. 2009b; Sritunyalucksana et al. 2006a). Because LSNV has been firmly linked to the sequence of WZSV 9 from China (Shi et al. 2016), the known geographical distribution of LSNV can be extended to include China (Taengchaiyaphum et al. 2020).

ICE was reported in LSNV-positive prawns from Thailand (Panphut et al. 2011).

Prevalence

Two independent studies on *P. monodon* samples from multiple farms in India reported a LSNV prevalence of 4% (3/72) and 5% (3/56) (Prakasha et al. 2007; Rai et al. 2009). In a third study on 81 *P. monodon* ponds from 2 districts in India, a 57% (46/81) prevalence of LSNV was reported (Sittidilokratna et al. 2009b). Out of 46 LSNV-positive ponds, LSNV infection was detected in prawns from 35/63 healthy ponds (55%) and 11/18 MSGS-affected ponds (61%) (Sittidilokratna et al. 2009b). A survey of multiple farms in India, found the highest prevalence of LSNV occurred in farmed juvenile *P. monodon* (49%; 17/35 prawns), followed by farmed juvenile *P. vannamei* (14%; 5/36 prawns) and farmed juvenile *P. merguiensis* (8%; 1/13 prawns) (Kumar et al. 2011). The same survey also examined pools of postlarvae samples from hatcheries and detected LSNV in 43% (7/16) of *P. monodon*, 20% (2/10) of *P. merguiensis* and 8% (1/12) of *P. vannamei* pools (Kumar et al. 2011).

LSNV has been detected in wild *P. monodon* broodstock (20%; 4/20 prawns) and wild juvenile *M. dobsoni* (20%; 3/15 prawns) (Kumar et al. 2011).

Mortalities

No reports of mortalities due to MSGS or natural infection with LSNV were found. However, experimental studies in *P. monodon* have reported mortalities ranging from 50–90%, 3–5 months after experimental exposure to MSGS-affected prawns or prawn tissue. For example, 90% of *P. monodon* fed with tissue homogenates from MSGS-affected prawns died and 100% were found to be LSNV-positive (Poornima et al. 2012). *P. monodon* which were cohabitated with MSGS-affected prawns had a 65% mortality rate, tested LSNV-positive and had a large size variation by the end of the 4.5 month experiment (Poornima et al. 2012). In another study, injection of *P. monodon* with lymphoid organ extracts from MSGS-affected prawns resulted in mortalities of 50%, a coefficient of

variation between 20–45% and darker colouration of the pleopods (Withyachumnarnkul 2005; Withyachumnarnkul et al. 2004).

Transmission

Membrane filtered lymphoid organ extracts from MSGS-affected *P. monodon* injected into healthy *P. monodon* induced MSGS (Anantasomboon et al. 2005; Withyachumnarnkul 2005; Withyachumnarnkul et al. 2004), suggesting that LSNV can be horizontally transmitted. In another experiment, lymphoid organ extracts from *P. vannamei* without MSGS-signs and co-cultured with MSGS-affected *P. monodon* caused MSGS when injected into healthy *P. monodon*, suggesting *P. vannamei* may be a susceptible species (Anantasomboon et al. 2005).

Experimental LSNV infections have been induced in *P. monodon* by ingestion of infected prawns (Poornima et al. 2012), cohabitation with infected prawns (Poornima et al. 2012; Wongprasert & Withyachumnarnkul 2009) and injection of viral preparations (Kumar et al. 2011; Panphut et al. 2011). Exposed prawns went on to develop signs of MSGS, such as slow growth (Panphut et al. 2011; Poornima et al. 2012; Wongprasert & Withyachumnarnkul 2009). Transmission of LSNV from broodstock to progeny occurs as the virus was detected in zoea and mysis stages of development and was shown to be transmitted from broodstock to offspring (Saksmerprome, Charoonnart & Flegel 2017; Wongprasert & Withyachumnarnkul 2009).

LSNV was detected by nested RT-PCR in the mud crab *S. serrata* following injection with a LSNV inoculum, indicating that other crustacean species are potential hosts of LSNV (Kumar et al. 2011). In a transmission study for ICE, tissue homogenates from ICE-positive *P. monodon* were injected into healthy *P. monodon* resulting in growth retarded prawns that were positive for ICE by RT-PCR (Panphut et al. 2011).

Mechanism of spread

Prawns were first found to be affected by MSGS in Thailand and the syndrome was soon identified in neighbouring Asian countries and East Africa (Anantasomboon et al. 2006; Chayaburakul et al. 2004; Sritunyalucksana et al. 2006a). It has been suggested that the emergence of MSGS started following large-scale importations of *P. vannamei* (Flegel 2004; Poornima et al. 2012) which are known to be hosts of LSNV (without developing the clinical signs of MSGS) (Kumar et al. 2011; Sakaew et al. 2008). These results could suggest that the emergence of MSGS in *P. monodon* in new areas might have occurred through exposure to *P. vannamei* carrying the necessary cause, LSNV. It has also been suggested that the sources of LSNV may have originated from exotic crustaceans that have been imported for aquaculture and for the ornamental aquarium trade (Poornima et al. 2012).

Infectious dose

The minimum infectious dose of LSNV required to cause symptoms of MSGS in prawns by experimental challenge or natural infection is not known. Approximately 1.0×10^7 DNA copies of LSNV was sufficient to result in 100% infectivity of healthy prawns 3–5 days post-injection (Ongvarrasopone, Chomchay & Panyim 2010).

Per os bioassays showed that LSNV was successfully transmitted to *P. monodon* fed with tissue homogenates from MSGS-affected prawns and resulted in 90% mortalities after 4.5 months post-infection (Poornima et al. 2012).

11.2.3 Pathogenesis

Pratoomthai et al. (2008) suggested that slow growth in the small prawns from MSGS-affected ponds may be due to a specific LSNV infection in the fasciculated zone and onion bodies of organ of Bellonci of the eyes. Growth retardation may also be related to the suppression of the release of crustacean hyperglycaemic hormone peptide by LSNV invasion in the zona fasciculata, consequently causing decreased glycogen breakdown in the hepatopancreas and persistent hypoglycaemia, resulting in growth stunting (Pratoomthai et al. 2012).

MSGS-affected prawns have been found co-infected with infectious hypodermal and haematopoietic necrosis virus (Rai et al. 2009). Prawns infected with LSNV can be co-infected with white spot syndrome virus (WSSV) or other viruses (Prakasha et al. 2007; Rai et al. 2009).

Tissue tropism

LSNV was detected in the lymphoid organ, gills, haemocytes, heart, hepatopancreas, pleopod, and neural tissues including the optic lobe, brain (supra-oesophageal ganglion), thoracic ganglion, abdominal ganglion and ventral nerve cord (Anantasomboon et al. 2005; Anantasomboon et al. 2008a; Chayaburakul et al. 2004; Flegel & Withyachumnarnkul 2005; Kumar et al. 2011; Sritunyalucksana et al. 2006a). Later, LSNV was found together with ICE in lymphoid organ, eyes and gills (Panphut et al. 2011).

Tissue titre

There have been few studies conducted on the load of LSNV in infected prawn tissues. Kumar et al. (2011) calculated by qRT-PCR the LSNV load (from gill and pleopods) in naturally infected juvenile *P. monodon* as 1.2×10^6 copies/µg of RNA, 2.9×10^5 copies/µg in *P. vannamei*, 4.7×10^4 copies/µg in *M. dobsoni* and 8.2×10^3 copies/µg in *P. merguiensis*. LSNV viral loads in naturally infected *P. monodon* broodstock using real-time reverse transcription loop-mediated isothermal amplification (qRT-LAMP) were found to be the highest in gill tissue followed by the lymphoid organ and haemolymph (Arunrut et al. 2014). LSNV loads in gills was 2 times higher than the load in the lymphoid organ and 3 times higher than the load in haemolymph (Arunrut et al. 2014).

11.2.4 Diagnosis

Clinical signs

As the cause of MSGS is uncertain, a working case definition was established to distinguish MSGS from slow growth caused by other agents. The MSGS-suspected prawn population should be RT-PCR positive for LSNV and must have a coefficient of variation (CV = standard deviation/mean) of >35% by weight and absence of hepatopancreatic parvovirus or other severe hepatopancreatic infections caused by known agents, while also complying with any 3 either:

- unusually dark colour
- average daily weight gain of less than 0.1 g/day at 4 months
- unusually bright yellow markings
- “bamboo-shaped” abdominal segments, and
- brittle antennae (Flegel 2008).

MSGS-affected prawns reached an average size of 12.5g with a very high CV for weight (30–80%), compared to the average non-MSGS prawn which weigh 24–40g after 4 months of culture (Chayaburakul et al. 2004).

LSNV has been detected in both growth retarded and healthy prawns (Kumar et al. 2011; Pratoomthai et al. 2008; Sittidilokratna et al. 2009b; Sritunyalucksana et al. 2006a). It has been noted, that the unusually bright yellow markings, "bamboo-shaped" abdominal segments or brittle antennae have not been evident in slow growth prawns from India, which are LSNV-positive by RT-PCR (Kumar et al. 2011).

Pathology

Retinopathy was observed exclusively in small LSNV-positive prawns collected from MSGS-affected ponds (Pratoomthai et al. 2008). Large LSNV-positive prawns from MSGS-affected ponds and LSNV-positive prawns from normal ponds did not suffer from retinopathy (Pratoomthai et al. 2008). Retinopathy included abnormally enlarged haemolymphatic vessels, haemocytic infiltration in the fasciculated zone and rupture of the membrane that separated the fasciculated zone from the overlying row of retinular cells (Pratoomthai et al. 2008). Further, LSNV was detected in the fasciculated zone of the eye and in onion bodies of the organ of Bellonci of the optic lobe in the small prawns of MSGS-affected ponds but not in those tissues of the large prawns from the MSGS-affected pond or from the normal-growth ponds, whether LSNV-positive or not (Pratoomthai et al. 2008).

Histopathology of MSGS-affected *P. monodon* also revealed the presence of large cytoplasmic inclusions in lymphoid organ spheroids and gill filaments (Anantasomboon et al. 2008a; Sritunyalucksana et al. 2006a).

Testing

LSNV can be detected using nucleic acid based methods such as RT-PCR (Sittidilokratna et al. 2009b; Sritunyalucksana et al. 2006a), nested RT-PCR (Prakasha et al. 2007; Sittidilokratna et al. 2009b), qRT-PCR (Kumar et al. 2011), reverse transcription loop-mediated isothermal amplification combined with a lateral flow dipstick (RT-LAMP-LFD) (Arunrut et al. 2011), and qRT-LAMP (Arunrut et al. 2014) using primers specific for the RNA dependent RNA polymerase. *In situ* hybridisation has also been used to test for LSNV (Sritunyalucksana et al. 2006a). RT-PCR and *in situ* hybridisation methods are also described to detect ICE (Panphut et al. 2011).

11.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments.

RNA interference (RNAi) based technology that has been shown to experimentally inhibit replication of LSNV may be developed as a tool to treat LSNV-infected prawn ponds (Ongvarrasopone, Chomchay & Panyim 2010; Saksmerprome, Charoonnart & Flegel 2017; Saksmerprome et al. 2013; Thammasorn et al. 2013).

11.2.6 Control

Control measures for MSGS in *P. monodon* have focused on the elimination of LSNV from prawn stocks (Thitamadee et al. 2016). LSNV has been added to the list of viruses to be excluded from domesticated specific pathogen-free (SPF) stocks of *P. monodon* in Thailand and it has been

recommended that prawn farmers avoid stocking LSNV-positive postlarvae to prevent MSGS (Arunrut et al. 2011; Panphut et al. 2011). It has been suggested that ICE should also be added to the list of excludable agents in SPF stocks (Panphut et al. 2011). It has been advised that to protect *P. monodon* from developing MSGS they should be reared separately from *P. vannamei*, particularly at the maturation and hatchery phases (Flegel 2004). This is because *P. vannamei* may be a host for LSNV (Kumar et al. 2011; Sakaew et al. 2008), which is a necessary but not sufficient cause of MSGS in *P. monodon*.

11.2.7 Impact of the disease

The most severe consequence of MSGS is its impact on the final harvest yield and value of the prawns due to reduced growth. Prawn farming in Thailand was severely affected during 2001–2002 due to the emergence of MSGS with reports that annual production volume was reduced by approximately 36% and resulted in a loss of US$300–400 million (Chayaburakul et al. 2004; Limsuwan 2006; Ongvarrasopone, Chomchay & Panyim 2010; Pratoomthai et al. 2008; Shinn et al. 2018a). The occurrence of MSGS in *P. monodon* in Thailand was reported as a major factor in causing Thai prawn farmers to convert to farming *P. vannamei* (Chayaburakul et al. 2004).

Although LSNV has been detected in wild prawns (Kumar et al. 2011), no reports were found about the impact of infection with LSNV on wild prawn populations.

11.2.8 Current biosecurity measures

MSGS was considered in the Prawn IRA 2009. At the time the Prawn IRA 2009 was prepared, MSGS was considered to present as a pattern of symptoms indicative of an infectious disease of unknown aetiology. It was determined that there was not enough information available to conduct a risk assessment. There are no current biosecurity measures specific for LSNV.

11.2.9 Conclusion

Whilst more information is available about the cause of MSGS compared to what was known when the Prawn IRA 2009 was completed, it still has not been determined exactly the role that LSNV and ICE play in development of MSGS. That is, the relationship between the two and their role in pathogenicity remains unclear. Although it is known that LSNV is a necessary but insufficient cause of MSGS. The department will continue to monitor developments in relation to the scientific knowledge and understanding of MSGS and review biosecurity measures as appropriate. Considering the absence of this information and the risk that MSGS poses given that Australia's prawn farming industry is primarily focused on *P. monodon*, the following risk assessment took a conservative approach and assumes that LSNV is the sole aetiological agent of MSGS (since it is a necessary component and exotic to Australia).

LSNV is present in exporting countries, is not present in Australia and is capable of causing adverse effects. In Australia, MSGS is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

11.3 Risk assessment

Based on chapter 4 and the technical information about LSNV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of LSNV meets Australia's ALOP are shown in Appendix F.

11.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for LSNV were that:

- This review is generic and therefore the entry assessment assumes that LSNV is present in all source countries.
- LSNV infects various penaeid prawn species of marketable size that are exported to Australia.
- Prevalence of LSNV can range from 4–57% in farmed prawns (Chayaburakul et al. 2004; Kumar et al. 2011; Prakasha et al. 2007; Rai et al. 2009; Sittidilokratna et al. 2009b). A single publication reported LSVN presence in wild prawns at a prevalence of 20% (Kumar et al. 2011).
- LSNV would be present primarily in the head (including hepatopancreas and gills) of infected prawns. LSNV has only been detected in the thoracic ganglion, abdominal ganglion and ventral nerve cord of the tail tissue.
- Assuming presence of LSNV is sufficient to cause MSGS, the viral load in LSNV-infected imported prawns is likely to be sufficient to cause infection in susceptible species.
- Post-harvest inspection may detect grossly abnormal prawns that are LSNV-positive and remove them before export. Prawns with mild gross signs or which are LSNV-positive without symptoms of MSGS would be unlikely to be detected.
- LSNV in imported prawns would be expected to survive freezing, storage and transport and remain infectious at the time of import.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of LSNV in imported prawns was estimated to be **high.**

11.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for LSNV were that:

- LSNV would be present primarily in the head of infected prawns (or associated wastes) that may enter the environment of the exposure groups.
- LSNV would be expected to be present in sufficient loads in imported prawns (or associated wastes) to cause infection in a susceptible species if exposed.
- LSNV in imported prawns (or associated wastes) is likely to persist and remain infectious at the point of exposure.
- The main aquaculture and wild-caught species in Australia that is susceptible to LSNV is *P. monodon*. Other species potentially susceptible to infection with LSNV are widespread in Australian waters.
- Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustaceans would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a

farm has not implemented standards of entry-level biosecurity for intake water that would exclude LSNV or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.

- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Species susceptible to LSNV may be present in research facilities and public aquaria.
- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley by recreational fishers. Prawn species susceptible to LSNV are present in Australian waters, and are likely to encounter imported prawns used as bait or berley. The host range for LSNV is narrow compared to hazards such as WSSV, therefore the likelihood of exposure is less than for those hazards.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to LSNV in imported prawns was estimated to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

11.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to LSNV in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

11.3.4 Consequence assessment

Partial likelihood of establishment and spread
The key points considered relevant when determining the partial likelihood of establishment and spread for LSNV were that:

- LSNV can be transmitted by ingestion of infected tissues, water and from broodstock to progeny.
- It is unknown if prawns that survive infection with LSNV can remain infectious.
- It is expected that susceptible species feeding on LSNV-infected prawns would receive an infectious dose.

- *P. monodon* which is the main prawn species farmed in Australia and a target species for fisheries, is susceptible to LSNV infection.
- Other LSNV hosts are present in Australia and include the crab *S. serrata* and *P. merguiensis.*
- The likelihood of LSNV establishment, following a given quantity of LSNV entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.
- If establishment of LSNV were to occur in the wild, spread to other populations and its natural geographic limits is less likely than for farmed or hatchery crustaceans because infected wild animals (particularly those clinically affected) are likely to be preyed upon by non-susceptible animals. The densities of susceptible and infected animals are lower, which reduces the likelihood of transmission. The host range of LSNV is much smaller than for other hazards such as WSSV which reduces the likelihood of spread.
- If LSNV were to establish in the wild, especially in waters around prawn farms, it may spread to farms through water. In the absence of effective biosecurity measures, wild infected prawns may be transferred into the farms through the inlet water channels. There are known species susceptible to LSNV, for example *S. serrata*, which are present in Australia and may be capable of entering farms through movement across short distances of land.
- If LSNV were to establish on a farm it could spread to neighbouring farms or wild populations through effluent water. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of LSNV be suspected and response measures initiated. However, LSNV is effectively transmitted through water, and farms which share a common water source with an infected population may be exposed to LSNV. Although it is unknown how long LSNV can persist in the water column and remain infectious.
- Spread from farms to wild populations or neighbouring farms via escaped prawns is reduced due to the systems in place on farms to prevent discharge of live animals.
- If LSNV were to establish in hatchery crustaceans, spread to wild crustaceans would be unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.
- Spread of LSNV from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae as prawn species cultured in Australia are susceptible to infection with LSNV and are unlikely to show clinical signs of disease at the time of transfer. LSNV is more likely to spread this way than hazards which have significant mortality as the broodstock may not be identified as diseased. A further complicating factor for LSNV is that broodstock may be infected with LSNV without showing signs of MSGS, but would be capable of spreading the virus to progeny who may go on to develop MSGS once transferred to the farm.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of LSNV in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Very low**.

Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of LSNV were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- Australia's main farmed prawn species, *P. monodon*, is susceptible to MSGS. Heavy production losses in farmed prawns are associated with MSGS.
- LSNV is not expected to impact wild fisheries in Australia. There have been no reports of declines in catch rates or associated mortalities in wild fisheries due to LSNV infection.
- LSNV establishment and spread would be expected to have a minor impact at the state or territory level on the life or health of susceptible animals.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There is no evidence that LSNV causes serious disease in non-penaeid species or freshwater crustaceans in the wild. The limited host range of LSNV would suggest the environmental effects of the introduction of LSNV in Australia would be minimal.
- Based on the absence of serious effects on wild prawn populations overseas, the environmental effects of LSNV establishment and spread are expected to be limited.
- The direct impact of LSNV establishment and spread on the environment is not expected to be discernible at any level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- MSGS is not listed as a notifiable disease by the WOAH and is not included in the *List of diseases in the Asia-Pacific*. However, MSGS is included on Australia's *National list of reportable diseases of aquatic animals* and state and territory governments would be expected to report on the agent.
- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating LSNV from wild crustacean populations is unlikely to be launched.
- If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely. However, disease may be undetected at first as MSGS is not associated with mortalities.
- If a movement regulated area were put in place for an outbreak of LSNV, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.
- Eradication of LSNV is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- A LSNV outbreak may affect the crab industries if movement regulated areas are put in place because crabs are possible hosts of LSNV.

- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- MSGS affected prawns would likely show severely retarded growth which would affect their marketability.
- LSNV establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. LSNV establishment and spread may result in loss of some crustacean export markets.
- If LSNV was to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product.
- The impacts of LSNV establishment and spread on international trade are likely to be minor at the local level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- No endangered Australian crustacean species, or those closely related, are susceptible to LSNV.
- The impacts of LSNV establishment and spread on biodiversity are not expected to be discernible at any level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Prawns that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of LSNV which may impact on social amenity.
- The social impacts of LSNV establishment and spread are expected to be minor at the local level.

Table 17 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of LSNV. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 17 Overall impact of establishment and spread of LSNV for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	State or territory	Minor	D
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	Local	Minor	B

Effects	Criteria	Level	Impact	Score
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	A
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	B

Conclusion
The overall impact of establishment and spread of LSNV was estimated to be **moderate**.

Determination of likely consequences of the outbreak scenario
The likely consequences of the outbreak scenario for LSNV in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Very low**.

11.3.5 Determination of partial annual risk
The partial annual risk of LSNV entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Very low**.
- Wild crustaceans—**Very low**.

11.3.6 Estimation of overall annual risk
The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with LSNV in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **low**.

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

11.4 Biosecurity measures
Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for LSNV in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 11.4.1, 11.4.2 and 11.4.3 present the factors considered and the conclusions reached.

11.4.1 Head and shell removal
When determining if head and shell removal would reduce the overall risk of LSNV to meet Australia's ALOP, the key points considered were:

- LSNV is present in a number of tissues which are found primarily in the head.

- Head and shell removal is expected to reduce the likelihood of entry of LSNV, however some LSNV is likely to remain in the tail.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Head and shell removal is expected to further reduce the likelihood of this occurring because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed for aquatic animals held in research or public aquaria.
- Whole uncooked prawns are preferred over other forms (including uncooked and shelled) of prawns for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal, applied was determined to be **negligible**.

11.4.2 Cooking

When determining if cooking would reduce the overall risk of LSNV to meet Australia's ALOP, the key points considered were:

- There are no reports of the effect of heating on LSNV infectivity.
- Given the effect of heating on LSNV infectivity is unknown, it is assumed that cooking may reduce, but not completely inactivate LSNV. Sufficient viable virus to cause disease will still be present. Therefore, cooking to attain a core temperature of at least 65°C is not expected to reduce the likelihood of entry.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **negligible**.

11.4.3 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of LSNV to meet Australia's ALOP, the key points considered were:

- Value-added products are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). The likelihood of entry of LSNV is expected to be the same as for head and shell removal. This is because it is not expected that the processing will further reduce the amount of viable LSNV in the product more than head and shell removal does.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

Conclusion

Based on this information, the overall restricted risk of imported frozen uncooked prawns which have been processed into a value-added product, was determined to be **negligible**.

12 Taura syndrome virus risk review

12.1 Background

Taura syndrome virus (TSV) is the aetiological agent of Taura syndrome (OIE 2021j). TSV is a member of the *Dicistroviridae* family (ICTV 2008; Mayo 2005). Susceptible host species include various penaeid prawns (Brock 1997b; Lightner 1996a).

Taura syndrome was first reported in farmed *Penaeus vannamei* in Ecuador in 1992 (Jimenez et al. 2000) and has since been detected in the Americas, Asia, some parts in East Africa and the Middle East.

Taura syndrome is listed as a disease notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Australia has a long history of passive surveillance and a strong system in place to detect incursions, TSV is considered exotic to Australia.

12.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of TSV is warranted.

12.2.1 Agent properties

TSV is an icosahedral, non-enveloped, single-stranded, positive-sense RNA virus that is 31–32nm in diameter (Bonami et al. 1997; Mari et al. 2002). TSV is formally classified by the International Committee on Taxonomy of Viruses as a member of the genus *Aparavirus*, in the family *Dicistroviridae* (ICTV 2008; Mayo 2005).

A single isolate of TSV had originally been shown as responsible for outbreaks of Taura syndrome in Ecuador (Bonami et al. 1997; Hasson et al. 1995; Mari, Bonami & Lightner 1998) and Hawaii (Bonami et al. 1997; Mari, Bonami & Lightner 1998). It now appears that at least four different strains or genotypic variants of TSV exist based on the gene sequence encoding capsid protein VP1, the largest of the three major structural proteins of the virus. These genotypes are the:

1) Americas group (Aranguren et al. 2013; Côté et al. 2008; Lightner 2011; Wertheim et al. 2009)
1) South-East Asian group (Nielsen et al. 2005; Wertheim et al. 2009)
2) Belize group (Erickson et al. 2005; Tang & Lightner 2005)
3) Saudi Arabia group (Tang et al. 2012).

There are several reports of the stability of TSV. Infectivity is retained after freezing at –70°C (Bonami et al. 1997; Overstreet et al. 1997; Tang, Wang & Lightner 2004) and –80°C (Hasson et al. 1995) and after freezing and storage at 0°C (Brock et al. 1995). TSV reportedly survives multiple freeze-thaw cycles in prawn tissues (Brock 1995; Brock et al. 1995; Hasson et al. 1995) and can remain infectious in water for up to 48 hours, in prawn head tissues for at least 14 days and in prawn tail tissues for at least 21 days at 27°C (Prior & Browdy 2002).

TSV can be inactivated by heat treatment at 121°C for at least 3.6 mins (Brock 1995). Crustacean products that have been subjected to heat treatment at 70°C for at least 30 minutes (or any

time/temperature equivalent that has been demonstrated to inactivate TSV) are considered safe based on the Safe commodity assessments for WOAH listed aquatic animal diseases (OIE 2016b, 2021i).

12.2.2 Epidemiology

Host range

Species which fulfil the criteria for listing as a species susceptible to infection (N= natural; E= experimental exposure) with TSV in accordance with chapter 1.5 of the WOAH *Aquatic animal health code* (WOAH Code) (WOAH 2022c) include:

- *Penaeus aztecus* [N, E] (Brock 1997b; Guzman-Saenz et al. 2009; Overstreet et al. 1997)
- *Penaeus ensis* [N] (Chang et al. 2004)
- *Penaeus monodon* [N, E] (Chang et al. 2004; Nielsen et al. 2005; Srisuvan, Tang & Lightner 2005)
- *Penaeus setiferus* [N, E] (Bonami et al. 1997; Guzman-Saenz et al. 2009; Overstreet et al. 1997)
- *Penaeus stylirostris* [N, E] (Overstreet et al. 1997; Robles-Sikisaka et al. 2002)
- *Penaeus vannamei* [N, E] (Lightner 1995).

Other host species shown to be susceptible to infection with TSV include (N= natural; E= experimental exposure):

- *Chelonibia patula* [E] (barnacle) (Overstreet, Jovonovich & Ma 2009)
- *Ergasilus manicatus* [E] (copepod) (Overstreet, Jovonovich & Ma 2009)
- *Octolasmis muelleri* [E] (barnacle) (Overstreet, Jovonovich & Ma 2009)
- *Penaeus chinensis* [E] (Lightner 1996b; Overstreet et al. 1997)
- *Penaeus merguiensis* [E] (Biosecurity Australia 2006; Ruangsri et al. 2005)
- *Penaeus monoceros* [E] (Ruangsri et al. 2005).

Also, TSV-positive RT-PCR and *in situ* hybridisation results have been reported in a few species (N= natural; E= experimental exposure), however no active infection has been demonstrated:, including:

- *Callinectes sapidus* [E] (crab) (Erickson et al. 1997a)
- *Cherax quadricarinatus* [E] (crayfish) (Biosecurity Australia 2006)
- *Cherax tenuimanus* [E] (marron) (Biosecurity Australia 2006)
- *Fundulus grandis* [E] (Gulf killifish) (Overstreet, Jovonovich & Ma 2009)
- *Gallus gallus domesticus* [E] (chicken) (Garza et al. 1997; Vanpatten, Nunan & Lightner 2004)
- *Larus atricilla* [N] (sea gull) (Garza et al. 1997; Vanpatten, Nunan & Lightner 2004)
- *Macrobrachium lanchesteri* [E] (Kiatpathomchai et al. 2008)
- *Macrobrachium rosenbergii* [N, E] (Biosecurity Australia 2006; Nielsen et al. 2005)
- *Palaemon styliferus* [E] (Kiatpathomchai et al. 2008)
- *Penaeus duorarum* [E] (Brock 1997b; Overstreet et al. 1997)
- *Penaeus indicus* [N] (Tang et al. 2012)

- *Penaeus japonicus* [N, E] (Brock 1997b; Nielsen et al. 2005)
- *Penaeus schmitti* [E] (Brock 1997b)
- *Sesarma mederi* [E] (crab) (Kiatpathomchai et al. 2008)
- *Scylla serrata* [E] (crab) (Kiatpathomchai et al. 2008)
- *Trichocorixa reticulate* [N] (water boatman) (Lightner 1995)
- *Uca vocans* [E] (crab) (Kiatpathomchai et al. 2008).

The susceptibility of host species and the virulence of the virus appear to vary with the TSV strain (Erickson et al. 2002; Erickson et al. 2005; Erickson, Zarain-Herzberg & Lightner 2002; Jiang et al. 2004; Srisuvan, Tang & Lightner 2005; Tang & Lightner 2005). For example, in the Prawn IRA 2009, *P. monodon* and *P. merguiensis* were considered to be susceptible to infection with TSV but largely resistant to significant clinical disease based on infection trials (Biosecurity Australia 2006). In the trials, *P. monodon* became infected with a Belize isolate of TSV via injection but not following *per os* challenge and not with a Thai isolate (by injection or *per os* exposure). *P. merguiensis* became infected with a Thai isolate of TSV by injection but not following *per os* challenge and was not tested for infection with a Belize isolate (Biosecurity Australia 2006). *P. vannamei* populations in Belize that were bred for resistance to the America TSV genotype suffered 90–100% mortality when infected with the Belize genotype (Erickson et al. 2005; Moss 2004). The susceptibility of host species may also vary with the life stage of the prawn. In *P. vannamei*, infection with TSV appears to have no impact on nauplii, mysis and early postlarval (PL) stages, but may exhibit as disease in animals from approximately PL12 onwards (Brock 1997b; Lightner 1996b).

Geographical distribution

Infection with TSV started causing significant production losses in farmed prawns in Ecuador in 1992 (Jimenez et al. 1992 cited in (Jimenez et al. 2000)) and it is now widespread in the Americas (Jimenez et al. 2000; Lightner 2011). TSV has also been reported from a number of Asian countries including: Myanmar (Network of Agriculture Centers in Asia-Pacific 2005; Nielsen et al. 2005), China (NACA & FAO 2004), Indonesia (Hanggono et al. 2005), Malaysia (NACA & FAO 2008), Philippines (Vergel et al. 2019), Republic of Korea (Do et al. 2006), Taiwan (Tu et al. 1999) and Thailand (Limsuwan 2003; Nielsen et al. 2005).

In addition, TSV has been reported in Eritrea in East Africa (Wertheim et al. 2009) and in Saudi Arabia (Tang et al. 2012). TSV was detected in a quarantine facility in Tahiti but was subsequently eradicated (Le Moullac et al. 2003).

Prevalence

TSV prevalence ranging from 0–100% have been reported in farmed prawns (Brock 1997b; Lightner 2011; OIE 2021j).

TSV prevalence of around 30% (sample size not reported) in farmed *P. vannamei* have been reported in Taiwan (Wang & Chang 2001). Prevalence of 23% (8/34), 36% (20/56) and 52% (32/62) have been reported from *P. vannamei* samples taken from farms in East Java, Indonesia in 2003, 2004 and 2005 respectively (Hanggono et al. 2005). In Thailand, TSV prevalence of 4% was reported from 163 *P. vannamei* postlarvae collected from hatcheries (Ruangsri et al. 2005). The same survey collected 192 juvenile *P. vannamei* from grow-out ponds and found a TSV prevalence of 7% (Ruangsri et al. 2005). In Mexico, TSV prevalence between 34–87% were reported in farmed *P. vannamei* collected

from prawn farms between 1995–1998 (Zarain-Herzberg & Ascencio-Valle 2001). A prevalence of 25% (15/60) has been reported in *P. vannamei* collected from prawn farms in the Philippines in 2019 (Vergel et al. 2019).

TSV prevalence of up to 32% have been reported in wild prawns (Morales-Covarrubias & Chavez-Sanchez 1999). TSV was found to be present at a prevalence of 8% (2/24) in wild *P. monodon* broodstock captured from southern Taiwan coastal waters in 2000 (Chang et al. 2004). Studies into the health status of wild hosts have reported TSV prevalence of 6.6% (12/180) in wild *P. setiferus* and *P. aztecus* collected between 2005–2006 from the Gulf of Mexico (Guzman-Saenz et al. 2009). Prevalence ranging from 27–32% in wild *P. vannamei* broodstock caught off the Mexican Pacific coast was reported (Morales-Covarrubias & Chavez-Sanchez 1999).

Mortalities

Cumulative mortalities due to TSV epizootics have ranged from 40% up to nearly 100% in farmed postlarvae, juvenile and sub-adult *P. vannamei* (Brock 1997b; Lightner 2011; Srisuvan, Tang & Lightner 2005). In countries such as Ecuador and Colombia, the onset of Taura syndrome in prawn farms was accompanied by sudden high mortalities, which reached accumulative mortality of up to 100% (Aranguren et al. 2013; Shrimp News International 1994). Similarly, TSV caused mortalities that exceeded 80% within 3 days of disease onset in farmed *P. vannamei* in Taiwan (Yu & Song 2000). Mortalities of 50–70% occurred in TSV-infected *P. indicus* farms in the Kingdom of Saudi Arabia (Tang et al. 2012). TSV has also been reported to cause mortalities in farmed *P. monodon* in Thailand (Srisuvan, Tang & Lightner 2005). Although, Taura syndrome mostly affects small juveniles (0.05g to <5g), larger prawns can be affected, particularly if they have not been previously exposed to TSV (Lightner 2011).

Transmission

Experimental infections have been induced by ingestion of infected prawns (Brock 1995), water-borne transmission (Prior et al. 2003), intramuscular injection of viral preparations (Hasson et al. 1995), incorporation of infected material into dietary brine shrimp (Overstreet et al. 1997) and cohabitation with infected prawns (Prior & Browdy 2002).

Although transmission from broodstock to progeny is thought to occur, it has not been shown to occur via oocytes (Lotz & Ogle 1997). In a single incident, female *P. stylirostris* inseminated with refrigerated Ecuadorian spermatophores imported to Tahiti produced offspring positive for TSV (Le Moullac et al. 2003). In another study, wild adult *P. vannamei* collected as broodstock were TSV-positive and within weeks produced postlarvae that also tested TSV-positive (Lightner 1995).

C. quadricarinatus and *C. tenuimanus* may act as vectors for TSV as neither could be infected with Thai or Belize isolates of TSV (Biosecurity Australia 2006). The virus was sequestered in the tissue of the challenged animals, but the virus did not form an active infection (Biosecurity Australia 2006). Both aquatic insects and seabirds may also play a role in the mechanical spread of TSV. Injection of *P. vannamei* with homogenised aquatic insects such as water boatman, *Trichocorixa reticulata*, collected near ponds undergoing an outbreak of Taura syndrome, or of homogenised faeces from seabirds feeding on prawns in affected ponds, has resulted in Taura syndrome (Lightner 1995). TSV also remains infectious to prawns following passage through the gut of chickens (*Gallus gallus domesticus*) and seagulls (*Larus atricilla*) (Garza et al. 1997; Vanpatten, Nunan & Lightner 2004).

Animals that survive infection during outbreaks of TSV can become chronically infected without showing clinical signs (Hasson et al. 1999; Krol et al. 1997; Lotz, Anton & Soto 2005; Lotz, Flowers & Breland 2003).

Mechanism of spread

The introduction of TSV into new areas has primarily been accredited to the movement of live animals, particularly broodstock, postlarvae (Brock 1995; Lightner 1995; Lightner et al. 1995; Nielsen et al. 2005; Tu et al. 1999; Yu & Song 2000) and genetic material, such as sperm (Le Moullac et al. 2003). It has been speculated that the international trade of frozen raw prawns may facilitate the introduction of prawn viruses into new areas. This may be through the inappropriate disposal of processing and retail wastes, the use of imported prawns for bait or the use of inadequately processed prawn feeds (Durand, Tang & Lightner 2000; Humphrey 1995; Lightner 1995; Lightner et al. 1995).

Infectious dose

The minimum infectious dose of TSV required to cause Taura syndrome in susceptible species by experimental challenge or natural infection is not known. However, *per os* bioassays showed that TSV has been successfully transmitted to *P. vannamei* by being fed once with 3% bodyweight of infected tissues (Argue et al. 2002; Cao et al. 2010). In other trials, *P. vannamei* has also been infected by feeding 7.5% bodyweight twice daily for 4 days (Erickson et al. 1997b), 10% bodyweight daily for 2 days (Côté & Lightner 2010), and 10% bodyweight for 3 days (Srisuvan et al. 2006; White et al. 2002).

In experimental infections in *P. vannamei,* a single injection of 50µL of a TSV inoculum (Belize and Colombia TSV isolates) containing a total of 2.5×10^6 TSV copies was sufficient to result in 100% mortality of the challenged populations within 5–6 days post-infection. This viral dose was reported to be routinely used at the University of Arizona Aquaculture Pathology Laboratory for TSV challenge tests (Aranguren et al. 2013).

12.2.3 Pathogenesis

There are three distinct but overlapping phases of TSV infection in penaeid prawns (Hasson et al. 1999). Following *per os* exposure, TSV is detectable in cells of the foregut, gills and general cuticle within 24 hours. The acute phase of infection is characterized by severe multifocal to diffuse necrosis of the cuticular epithelium and sub-cutis of the foregut, gills, appendages, general body surface, and, to a lesser extent, the hindgut (Hasson et al. 1999). This phase lasts up to 7 days. The transition phase begins on about the fourth day post-exposure and lasts for approximately 5 days. Multiple, multifocal, melanised cuticular lesions are present. Some acute phase lesions are evident, but there is also the beginning of lymphoid organ spheroid formation, with the uptake of TSV in the cells of the lymphoid organ tubules. If prawns survive this stage, they moult, shedding their melanised cuticle to enter the chronic phase of infection. The chronic phase starts at about 6 days post-exposure, overlapping with the previous two phases. Chronically infected animals display no clinical signs; however, there is marked lymphoid organ hypertrophy due to spheroid formation (Hasson et al. 1999). As determined by bioassay, the virus can remain infectious in survivors of Taura syndrome for at least 8 months after an outbreak (Krol et al. 1997). Recurrence of disease outbreaks in chronically infected animals is usually precipitated by environmental stressors such as temperature and salinity changes following heavy rain or drought (Edwards 1998; Lotz, Anton & Soto 2005).

Tissue tropism
TSV is reported to infect the cytoplasm of cells from tissues of ectodermic and mesodermic origin, including the sub-cuticular epithelium of the body, appendages, gills, mouth, oesophagus, stomach and hindgut (Hasson et al. 1995; Lightner et al. 1995). The antennal gland tubule epithelium is occasionally affected (Lightner et al. 1995). The sub-cuticular connective tissue and adjacent striated muscle fibres basal to cuticular epithelial cells are also sometimes involved (Lightner et al. 1995). In chronic infections, TSV is concentrated in the lymphoid organ, but may also be present in other tissues (Tang, Wang & Lightner 2004).

Tissue titre
Few studies have examined the titre of TSV in infected prawn tissues. Nunan et al. (2004) fed juvenile *P. vannamei* (mean weight approximately 3g) with minced TSV-positive prawn tissues (5% bodyweight per day for 2 days) and reported 10^6–10^{10} TSV genome copies/g of host tissue in tails, tail fans, gills, pleopods and heads of the resulting infected prawns (Nunan, Tang-Nelson & Lightner 2004). In similar experiments, Tang et al. (2004) reported 10^6–10^8 TSV genome copies/µg of RNA in both gills and pleopods of acute and chronically infected *P. vannamei*. In the chronically infected *P. vannamei*, there was a higher number of TSV copies in the lymphoid organ (10^8–10^9 TSV genome copies/µg of RNA) compared to the gills and pleopods (Tang, Wang & Lightner 2004). Aranguren et al. (2013) challenged *P. vannamei* specific pathogen free (SPF) prawns (average weight 3g) with different TSV isolates (inoculum injection of 2.5×10^6 TSV copies) and found 9.6×10^9, 1.7×10^{10}, and 2.8×10^{10} TSV copies/µg RNA in pleopods of the prawns challenged with Hawaii, Belize and Colombia TSV isolates, respectively. The prawns showed 100% mortality after 5–6 days post-infection with the Belize and Colombia isolates, and 97% mortality after 8 days post-infection with the Hawaii TSV isolate.

12.2.4 Diagnosis

Clinical signs
Most clinical signs associated with Taura syndrome are non-specific. In *P. vannamei,* gross signs have been documented in all life stages except eggs, zygotes and larvae (Lightner 2011). Prawns in the acute stage of infection are often lethargic, anorexic, ataxic, and, as they are typically in late stages of the moult cycle, soft-shelled (Brock 1995; Lightner et al. 1995; Yu & Song 2000). Prawns with Taura syndrome may appear either red or blue due to the expansion of chromatophores (Chamberlain 1994; Lightner et al. 1995). Although most diseased prawns die within one week, some can survive and become chronic carriers (Brock 1995; Lightner et al. 1995). Surviving prawns show multiple melanised cuticular lesions that pale or disappear following moult; some de-pigmented foci may remain in some animals (Brock 1995; Lightner et al. 1995). The susceptibility of prawns to clinical disease has been shown to vary with the TSV strain (Erickson et al. 2002; Erickson et al. 2005; Erickson, Zarain-Herzberg & Lightner 2002; Jiang et al. 2004; Srisuvan, Tang & Lightner 2005; Tang & Lightner 2005).

TSV has also been detected in a wide range of non-penaeid crustaceans but without active infection and therefore no clinical signs of disease were present.

Pathology
Taura syndrome can be diagnosed in the acute and chronic phases using histological methods (OIE 2021j). TSV-induced pathology is pathognomonic with haematoxylin and eosin stained preparations

showing multifocal areas of necrosis, with a 'peppered' or 'buckshot-riddled' appearance, in the sub-cuticular epithelium of the body, appendages, gills, hindgut, and foregut (Lightner 2011). These pathognomonic bodies correspond to cytoplasmic remnants of necrotic cells, that show as eosinophilic to pale basophilic spherical bodies, together with pyknotic or karyorrhectic nuclei (Lightner 2011). The transition or recovery phase of Taura syndrome presents a decrease in the amount and severity of typical acute phase cuticular lesions together with infiltration and accumulation of haemocytes at the sites of necrosis. In chronic infections, the only lesion typically presented by infected prawns is the presence of an enlarged lymphoid organ with multiple spheroids (Lightner 2011).

Testing

Chapter 2.2.7 of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH Manual) (WOAH 2022h) provides details of the methods currently available for targeted surveillance and diagnosis of TSV, in addition to which tests are recommended for targeted surveillance to declare freedom from infection with TSV.

RT-PCR or qRT-PCR are recommended methods for targeted surveillance to declare freedom from TSV (WOAH 2022h). The WOAH Manual also advise the demonstration of pathognomonic TSV-induced lesions in the cuticular epithelium by histology (with or without confirmation by *in situ* hybridisation with TSV-specific DNA probes) as a suitable method when investigating acute mortality episodes as part of a targeted surveillance programme (WOAH 2022h).

12.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments (WOAH 2022h).

12.2.6 Control

Control measures for Taura syndrome are primarily aimed at preventing the introduction of the virus into susceptible populations. TSV-resistant stocks of *P. stylirostris* and *P. vannamei* have been generated and used in prawn farms in the Americas and South-East Asia. The development of SPF prawn stocks of *P. vannamei* and *P. stylirostris* has proven to be one of the most successful husbandry practices for the prevention and control of infection with TSV (OIE 2021j). Most commercial stocks of *P. vannamei* used in Asia are now highly tolerant to TSV. Its impact on this species has declined due to the introduction of tolerant stocks and implementation of good biosecurity (Flegel 2012). Other general husbandry practices and disease control and management practices include the application of PCR assays for pre-screening of broodstock and/or their spawned eggs/nauplii and discarding those that test positive for TSV, as well as the disinfection of eggs and larvae (OIE 2021j).

12.2.7 Impact of the disease

Infection with TSV has caused losses of US$1.5–3 billion (US$1–2 billion in Americas and US$0.5–1 billion in Asia) for the prawn farming industry around the world since its emergence (Lightner 2011). TSV infections in Ecuador resulted in a 30% reduction in prawn production during 1992 with losses estimated at US$400 million (Lightner 1996a; Shinn et al. 2018b). Two Peruvian sites encountered a US$2.5 million loss following establishment of an infection in 1993 (Talavera & Varas, 2001 cited in (Shinn et al. 2018b)). Prawn production in Honduras decreased by 18% in 1994, 31% in 1995 and 25% in 1996 due to TSV infections and resulted in a 18% drop in labour costs (Shinn et al. 2018b). TSV

detected in Panama in 1996 caused a 30% decrease in prawn production (approximately 285 million tonnes) (Morales et al. 2001 cited in (Shinn et al. 2018b)). In Mexico, TSV caused a decrease in prawn production in 2007 that resulted in losses estimated at US$15 million (López-Téllez et al. 2019). Reviews on large scale economic losses in aquaculture due to disease have not reported recent losses resulting from TSV infection (Shinn et al. 2018a; Shinn et al. 2018b). This may be because of the widespread use of TSV resistant stocks of *P. vannamei*.

There are no data to suggest that TSV infection has impacted wild prawn populations. Although TSV was found in wild prawns in Ecuador (Brock 1995), Mexican Pacific coast (Morales-Covarrubias & Chavez-Sanchez 1999) and Taiwan (Chang et al. 2004) there was reportedly no decline in wild broodstock levels. There was no decline in wild prawn catches reported following the outbreak of Taura syndrome in nearby Texan prawn farms in 1995 (Campbell 1996).

12.2.8 Current biosecurity measures

The Prawn IRA 2009 determined the unrestricted risk associated with TSV to be low and therefore biosecurity measures were necessary (Biosecurity Australia 2009).

Current biosecurity measures which manage risks for TSV are:

- demonstration of source population freedom
- cooking
- highly processed prawn products (dumpling and dim sum type-products)
- breaded, battered or crumbed prawns
- head and shell removal (last segment and tail fan excluded).

12.2.9 Conclusion

TSV is present in exporting countries, is not present in Australia and is capable of causing adverse effects in Australia. In Australia, Taura syndrome is a nationally notifiable disease and biosecurity measures are in place. Based on the preceding information, risk assessment is warranted.

12.3 Risk assessment

Based on chapter 4 and the technical information about TSV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of TSV meets Australia's ALOP are shown in Appendix F.

12.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for TSV were that:

- This risk review is generic and therefore the entry assessment assumes that TSV is present in all source countries.
- TSV infects various penaeids and has been found by PCR in caridean prawn species of marketable size that are exported to Australia.

- Prevalence of TSV can range from 0–100% in farmed prawns (Brock 1997b; Lightner 2011; Tang et al. 2017; Wang & Chang 2001) and from 0–32% in wild prawn populations (Chang et al. 2004; Guzman-Saenz et al. 2009; Morales-Covarrubias & Chavez-Sanchez 1999).
- TSV would be present in the whole body of infected prawns.
- The viral load of TSV in infected imported prawns is likely to be sufficient to cause infection in susceptible species.
- Post-harvest inspection may detect grossly abnormal prawns that are TSV-positive and remove them before export. Prawns with mild gross signs, those with no clinical signs or prawns which have recently moulted would be unlikely to be detected.
- TSV in imported prawns is expected to survive freezing, storage and transport and remain infectious at the time of import.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of TSV in imported prawns was estimated to be **high**.

12.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for TSV were that:

- TSV would be present in the whole body of infected prawns or in associated wastes that may enter the environment of the exposure groups.
- TSV would be expected to be present in sufficient loads in imported prawns (or associated wastes) to cause infection in a susceptible species if exposed.
- TSV in imported prawns (or associated wastes) is likely to persist and remain infectious in water at the point of exposure for an extended period.
- Important aquaculture and wild-caught species in Australia, including *P. monodon* and *P. merguiensis* are susceptible to TSV infection. Although susceptibility is TSV strain, species and exposure route dependent. Other crustacean species, such as *C. quadricarinatus, P. indicus, M. rosenbergii* and *P. japonicus* are widespread in Australian waters and have been reported to sequester TSV but do not show signs of active infection.
- Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustaceans would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a farm has not implemented standards of entry-level biosecurity for intake water that would exclude TSV or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.
- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume

of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Species susceptible to TSV may be present in research facilities and public aquaria.

- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley. Species susceptible to TSV are present in Australian waters and are likely to encounter imported prawns used as bait or berley. The host range of TSV is narrower than that of white spot syndrome virus (WSSV), therefore the likelihood of exposure is less than for WSSV, but species susceptible to TSV are widespread.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to TSV in imported prawns was estimated to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

12.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to TSV in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

12.3.4 Consequence assessment

Partial likelihood of establishment and spread
The key points considered relevant when determining the partial likelihood of establishment and spread for TSV were that:

- TSV can be transmitted via ingestion of infected tissues and from broodstock to progeny. TSV can also be transmitted via water where it can remain infectious for up to 48 hours.
- It is expected that susceptible host animals feeding on TSV-infected prawns would receive an infectious dose.
- Prawns that survive TSV infection can remain infectious and become sources of the virus.
- Important aquaculture and wild-caught species in Australia, including *P. monodon* and *P. merguiensis* are susceptible to TSV infection. Although susceptibility is TSV strain, species and exposure route dependent.
- Other crustacean species, such as *C. quadricarinatus*, *P. indicusm, M. rosenbergii* and *P. japonicus* are widespread in Australian waters and have been reported to sequester TSV but do not show signs of active infection.
- Species present in Australia that may act as TSV vectors include crabs, water boatman and seagulls.

- The likelihood of TSV establishment, following a given quantity of TSV entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.
- If one or more index cases of TSV were to occur, establishment in the directly exposed wild crustaceans is less likely than for farmed or hatchery crustaceans because infected wild animals (particularly those clinically affected) are likely to be prey for non-susceptible animals. The densities of susceptible and infected animals are also much lower which reduces the opportunities for transmission. The range of species susceptible to TSV and present in Australia is much smaller than for other hazards, such as WSSV. Additionally, of those species present in Australia such as *P. monodon* and *P. merguiensis*, their susceptibility to TSV is dependent upon the method of exposure and the strain of TSV, therefore it is considered less likely that TSV would establish and spread to its natural geographic limits compared to other hazards such as WSSV.
- If TSV were to establish in the wild, especially in waters around prawn farms, it may spread to farms due to being transmissible through water, however it would depend on the TSV strain as to whether Australian species would be susceptible to TSV via water transmission. In the absence of effective biosecurity measures, wild infected prawns may be transferred into the farms through the inlet water channels. TSV may also be spread by vectors present in Australia, for example *S. serrata*, which can enter farms through movement across short distances of land.
- If TSV were to establish on a farm it could spread to neighbouring farms or wild populations through effluent water. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of TSV be suspected and response measures initiated. However, TSV is effectively transmitted through water, and susceptible animals which share a common water source with an infected population may be exposed to TSV. TSV can remain infective in the water column for some time. Further, if the TSV strain did not cause clinical disease, the detection of the outbreak on the farm may be delayed compared to outbreaks of other hazards which cause significant clinical disease such as strains of *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease.
- Spread from farms to wild populations or neighbouring farms via escaped prawns is possible, although likelihood is reduced due to the systems in place on farms to prevent discharge of live animals. TSV could also be spread from farms to wild populations via seabirds scavenging dead or moribund prawns and dropping them in unaffected waters. TSV has also been demonstrated to remain infectious after passing through the gut of seagulls.
- If TSV were to establish in hatchery crustaceans, spread to wild crustaceans would be unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.
- Spread of TSV from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae as prawn species cultured in Australia are susceptible to some strains of TSV. TSV is more likely to spread this way than hazards which have significant clinical signs or high mortality as the broodstock and postlarvae may not be identified as diseased.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of TSV in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**Moderate**.

- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Very low**.

Determining adverse impacts resulting from the outbreak scenario
The key points considered relevant when determining the adverse impacts resulting from establishment and spread of TSV were that:

Direct effects
The effect on the life or health (including production effects) of susceptible animals

- Australia's main farmed prawn species are susceptible to TSV. There is high morbidity and mortality associated with infection, although this is impacted by the TSV strain, species of prawn and route of exposure.
- TSV is not expected to impact wild fisheries in Australia. There have been no reports of declines in catch rates or associated mortalities in wild fisheries due to TSV infection.
- Based on the impacts in the Americas and Asia from TSV infection, TSV establishment and spread in Australia would be expected to cause minor impacts at the state or territory level on the life or health of prawns.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- TSV has been detected in wild prawn species of which susceptible species are distributed in Australian waters. However, in other regions where TSV is endemic, there have been no reports of clinical disease or mortalities in the wild due to TSV.
- TSV has been detected in crayfish which are found in Australia but they are proposed to act as vectors rather than susceptible species so no effect on them is anticipated.
- Based on the absence of serious direct effects on the environment in areas where TSV is endemic, the effect of TSV establishment and spread on the environment is expected to be minor at the local level.

Indirect effects
The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with TSV is listed as a notifiable disease by the WOAH and is included on Australia's *National list of reportable diseases of aquatic animals*. State and territory governments would be expected to report on the agent.
- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating TSV from wild crustacean populations is unlikely to be launched.
- If infected animals were considered confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely.
- If a movement regulated area were put in place for an outbreak of TSV, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.
- Eradication of TSV is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- A TSV outbreak may affect freshwater and marine crustacean industries if movement regulated areas are put in place because some of these species are vectors for TSV.
- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- TSV infected prawns would likely show gross signs which may affect their marketability.
- TSV establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- TSV is a WOAH-listed disease. Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. TSV establishment and spread may result in loss of some crustacean export markets due to importing country biosecurity requirements.
- If TSV were to become established, Australia could use zoning to maintain or gain access to international markets for crustaceans including prawns and, if required, non-viable product.
- The impacts of TSV establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- No endangered Australian crustacean species are currently known to be susceptible to infection with TSV. However, TSV is capable of mutating into more virulent strains (Dhar et al. 2004). If a new strain were able to cause clinical disease in the critically endangered *C. tenuimanus* it could result in a significant impact on the survival of an already endangered species.
- In light of the uncertainty surrounding the susceptibility of *C. tenuimanus* to infection with TSV, a conservative approach has been adopted when considering the susceptibility of native species, particularly those that are endangered or threatened, and it is assumed they are susceptible.
- The impact of TSV establishment and spread on the biodiversity of the environment is considered to be minor at the national level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Freshwater and marine crustaceans that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of TSV which may impact on social amenity.
- The social impacts of TSV establishment and spread are expected to be minor at the local level.

Table 18 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of TSV. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 18 Overall impact of establishment and spread of TSV for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	State or territory	Minor	D
	The environment (native animals/plants, and non-living environment)	Local	Minor	B
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	National	Minor	E
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	B

Conclusion
The overall impact of establishment and spread of TSV was estimated to be **moderate**.

Determination of likely consequences of the outbreak scenario
The likely consequences of the outbreak scenario for TSV in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**Moderate**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Very low**.

12.3.5 Determination of partial annual risk

The partial annual risk of TSV entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Very low**.
- Wild crustaceans—**Very low**.

12.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with TSV in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **low**.

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

12.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for TSV in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 12.4.1, 12.4.2 and 12.4.3 present the factors considered and the conclusions reached.

12.4.1 Head and shell removal

When determining if head and shell removal would reduce the overall risk of TSV to meet Australia's ALOP, the key points considered were:

- Head and shell removal is not expected to reduce the likelihood of entry of TSV. This is because TSV infects tissues throughout the whole prawn. Whilst head and shell removal would reduce the viral load in the prawn, sufficient TSV to cause infection in a susceptible species following exposure is expected to remain.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Head and shell removal is expected to further reduce the likelihood of this occurring because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed for aquatic animals held in research or public aquaria.
- Whole uncooked prawns are preferred over other forms (including uncooked and shelled) of prawns for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal, applied was determined to be **negligible**.

12.4.2 Cooking

When determining if cooking would reduce the overall risk of TSV to meet Australia's ALOP, the key points considered were:

- Crustacean products that have been subjected to heat treatment at 70°C for at least 30 minutes (or any time/temperature equivalent that has been demonstrated to inactivate TSV) are considered safe based on the *Safe commodity assessments for* WOAH *listed aquatic animal diseases* (OIE 2016b; WOAH 2022i).
- Given the length of time required to inactivate TSV in a prawn cooked to a core temperature of 65°C is well outside the time what would be expected for commercial cooking of prawns, it is assumed that cooking may reduce, but not completely inactivate TSV in imported prawn tissue. Sufficient viable virus to cause disease will likely still be present. Therefore, cooking to a core temperature of 65°C is not expected to reduce the likelihood of entry.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the

nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.

- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **negligible**.

12.4.3 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of TSV to meet Australia's ALOP, the key points considered were:

- Value-added products are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). The likelihood of entry of TSV is expected to be the same as for head and shell removal. This is because it is not expected that the processing will further reduce the amount of viable TSV in the product more than head and shell removal does.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

Conclusion

Based on this information, the overall restricted risk of imported frozen uncooked prawns which have been processed into a value-added product, was determined to be **negligible**.

13 *Vibrio parahaemolyticus* (strains causing acute hepatopancreatic necrosis disease) risk review

13.1 Background

Vibrio parahaemolyticus strain Vp AHPND is the aetiological agent of acute hepatopancreatic necrosis disease (AHPND), a bacterial disease of farmed penaeid prawns (OIE 2021b). AHPND is characterised by sudden mass mortalities that commonly occur within 30–35 days of stocking grow-out ponds with postlarvae or juveniles (OIE 2021b).

Vp AHPND carries a plasmid with genes that encodes two Pir-like toxins (*Photorhabdus* insect-related), PirA and PirB (OIE 2021b). Bacteria other than *V. parahaemolyticus* have been found to have Pir toxin genes and to cause AHPND-like symptoms (Ahn et al. 2017; Dong et al. 2017a; Dong et al. 2017b; Durán-Avelar et al. 2018; Han et al. 2016; Kondo et al. 2015; Liu et al. 2020a; Liu et al. 2018a; Muthukrishnan et al. 2019; Restrepo et al. 2018; Vicente et al. 2020). However, Australia adheres to the definition of AHPND in the World Organisation for Animal Health (WOAH, formerly OIE) *Aquatic animal health code* (WOAH Code) Article 9.1.1. and this definition is used for the purposes of this risk review.

> For the purposes of the Aquatic Code, acute hepatopancreatic necrosis disease (AHPND) means infection with strains of *Vibrio parahaemolyticus* (Vp AHPND), of the family *Vibrionaceae*, that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB (WOAH 2022c).

AHPND has also been referred to as early mortality syndrome (EMS) in early publications, but this general designation caused confusion due to other causes of early mortality (Flegel & Lo 2014; Tran et al. 2013b).

Susceptible host species include various penaeid prawns (de la Peña et al. 2015; Lightner et al. 2012a). AHPND was first reported in farmed prawns from China and Vietnam in 2010 and has since been detected in several Asian countries and other parts of the world (Flegel 2012; Lightner et al. 2012a; OIE 2021b).

AHPND is listed as a disease notifiable to the WOAH (OIE 2021c) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Australia has reported hepatopancreatitis in prawns to the WOAH but it did not satisfy the WOAH case definition of AHPND as it was caused by a *Vibrio* species other than Vp AHPND (as identified using whole genome sequencing) (OIE 2016a). Australia has a long history of passive surveillance and a strong system in place to detect incursions, AHPND is considered exotic to Australia.

13.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of Vp AHPND is warranted.

13.2.1 Agent properties

V. parahaemolyticus is a gram-negative, halophilic bacterium that belongs to the *Vibrionaceae* family, and is ubiquitous in marine and brackish water environments. It is rod-shaped with a single polar flagellum that makes it motile in liquid medium (Baumann and Schubert 1984 cited in (Su & Liu 2007)).

V. parahaemolyticus strains causing AHPND are uniquely virulent to prawns because they carry a virulence plasmid (pVA1, ~70 kbp), which contains the PirA and PirB genes that encode homologues of the Pir binary toxins, PirA and PirB (Gomez-Gil et al. 2014; Gomez-Jimenez et al. 2014; Han, Tang & Lightner 2015; Han et al. 2015b; Kondo et al. 2014; Lai et al. 2015; Lee et al. 2015; Yang et al. 2014). Toxins PirA and PirB induce cell death and are responsible for the primary pathology in affected prawns (Tran et al. 2013a; Tran et al. 2013b).

Plasmid pVA1 is stably inherited via a post-segregational killing system during bacterial replication and disseminated between different *Vibrio* species through horizontal gene transfer (conjugation, transposition, homologous recombination or natural genetic transformation) (Carrillo-Méndez et al. 2019; Dong et al. 2019a; Dong et al. 2019b; Lee et al. 2015). Natural absence or experimental deletion of pVA1 plasmid abolishes the ability of Vp AHPND strains to cause disease (Lee et al. 2015; Tinwongger et al. 2016). Genetic variability in the plasmid sequences has been reported which has led to a description of a Mexican-type and an Asian-type (Han et al. 2015b). Phylogenetic analysis has also shown that Vp AHPND isolates can be clearly differentiated into distinct clusters specific to different regions (Fu et al. 2017).

Novel isolates of *V. parahaemolyticus* that contain the PirA and PirB toxin genes but that do not cause AHPND in prawns have been reported. An atypical isolate of *V. parahaemolyticus* that contained the full-length PirA and PirB toxin genes was isolated from healthy *Penaeus vannamei* (Kanrar & Dhar 2018b). Conversely, another novel isolate of *V. parahaemolyticus* was reported to cause mortalities (without typical AHPND pathology) in *P. vannamei* but it also did not produce PirA or PirB toxins despite carrying the genes (Vicente et al. 2020).

Attempts to transmit AHPND using infected frozen prawns have been unsuccessful (Tran et al. 2013a), suggesting that Vp AHPND is inactivated by freezing and thawing. Although, it has been reported that *V. parahaemolyticus* can survive refrigeration and frozen storage ((FSANZ 2016) citing (ICMSF 1996; Oliver et al. 2013)). However, in general, *Vibrio* species are known to be sensitive to freezing, refrigeration, heating and common disinfectants (OIE 2021b; Vanderzant & Nickelson 1972). *V. parahaemolyticus* in seafood is known to be sensitive to freezing (–18 to –24°C for several weeks) and heating (55°C for 5 mins and 80°C for 1 min); where culturable cells were reduced to non-detectable levels (Andrews, Park & Chen 2000; Muntada-Garriga et al. 1995; Su & Liu 2007; Vanderzant & Nickelson 1972). Culturable cells of *V. parahaemolyticus* are also reduced following refrigeration at 4°C, but not to non-detectable levels (Su & Liu 2007). However, it has been reported that *V. parahaemolyticus* strains are able to enter a viable but non-culturable state when stored under refrigeration (Baffone et al. 2003) which may impact these results. *V. parahaemolyticus* which

is viable but in a non-culturable state shows metabolic activities and under appropriate conditions it is able to recover from this dormant state, became metabolically active, fully culturable and it can reactivate its pathogenic potential (Baffone et al. 2003). Crustacean products that have been subjected to heat treatment at 100°C for at least 1 minute (or any time/temperature equivalent that has been demonstrated to inactivate Vp AHPND) are considered safe based on the Safe commodity assessments for WOAH listed aquatic animal diseases (OIE 2016b, 2021a).

Vp AHPND can live independently and persist in marine environments, sediments and biofilms (Thitamadee et al. 2016). The sediment of prawn-farming ponds have been proposed as a reservoir of Vp AHPND (Yang et al. 2019). Vp AHPND can tolerate a wide range of salinities; causing infection and mortalities in salinities of 5, 10, 15 and 20 ppt (Schofield et al. 2020).

V. parahaemolyticus can grow in sodium chloride concentrations ranging from 0.8–11% (Karunasagar et al. 1987). *V*. *parahaemolyticus* has been reported to survive in filtered estuarine water for 9 days and in filtered seawater for over 18 days at ambient temperature (28 ± 2°C) (Karunasagar et al. 1987). *V. parahaemolyticus* can also survive in freshwater ecosystems (Sarkar et al. 1985; Venkateswaran et al. 1989). Although, its distribution is transient, being mostly isolated from sediment or water in the summer months and often found in association with biological hosts, mainly fish and plankton (Sarkar et al. 1985). It has been reported that under conditions of estuarine salinity the adsorption of *V. parahaemolyticus* on plankton or chitin-containing materials occurs more efficiently, which improves its survival at low temperatures (Colwell et al. 1984).

13.2.2 Epidemiology

Host range

Species which fulfil the criteria for listing as a species susceptible to infection (N= natural; E= experimental exposure) with Vp AHPND in accordance with chapter 1.5 of the WOAH Code (WOAH 2022c) include:

- *Penaeus monodon* [N] (de la Peña et al. 2015; Eshik et al. 2018; Lightner et al. 2012a)
- *Penaeus vannamei* [N, E] (de la Peña et al. 2015; Lightner et al. 2012a; Tran et al. 2013b).

Species for which there is incomplete evidence for listing as susceptible to infection (N= natural; E= experimental exposure) include:

- *Artemia franciscana* [E] (Kumar et al. 2018; Kumar et al. 2019; Muthukrishnan et al. 2019)
- *Exopalaemon carinicauda* [E] (Ge et al. 2018; Ge et al. 2017)
- *Macrobrachium rosenbergii* [N, E] (Kumar et al. 2018; Tun et al. 2017)
- *Penaeus chinensis* (OIE 2021b)
- *Penaeus japonicus* (OIE 2021b)
- *Penaeus semisulcatus* [N, E] (Megahed 2018).

Species for which Vp AHPND-positive PCR results have also been reported include (N= natural exposure):

- Bait worms (polychaetes) [N] (FAO 2017; Thitamadee et al. 2016; Tran 2018)
- Bivalves (oysters, clams) [N] (Thitamadee et al. 2016)

- *Cherax quadricarinatus* [E] (Powers et al. 2021).

Susceptible stages include postlarvae, juveniles and adults (de la Peña et al. 2015; Deris et al. 2020; Joshi et al. 2014b; Nunan et al. 2014; OIE 2021b; Soto-Rodriguez et al. 2015; Tran et al. 2013b).

There is conflicting evidence about the susceptibility of *M. rosenbergii* to Vp AHPND. One study has reported mortalities following exposure (Kumar et al. 2018) and another has not (Schofield et al. 2020). It is speculated that the size of the *M. rosenbergii* used in the second study may explain why there was no mortality or histological evidence of AHPND.

Vp AHPND has been detected in live broodstock feeds such as polychaetes and bivalves (FAO 2017; Thitamadee et al. 2016; Tran 2018).

Cherax quadricarinatus cohabited with AHPND-affected *P. vannamei* did not develop histopathological signs of disease. However, a small subset did test positive by PCR, indicating that this species is likely resistant to disease caused by Vp AHPND but may act as a carrier (Powers et al. 2021).

Geographical distribution

AHPND was detected in China and Vietnam in 2009–10 (Flegel 2012; Lightner et al. 2012a) as well as in Bangladesh (Eshik et al. 2018), Myanmar (NACA, OIE-RRAP & FAO 2016; Tun et al. 2017), Malaysia (Chu et al. 2016), Philippines (de la Peña et al. 2015), Republic of Korea (Han et al. 2020), Taiwan (OIE 2019a) and Thailand (Flegel 2012; Joshi et al. 2014b; Kondo et al. 2014). An isolated event of AHPND occurred in Japan, after an introduction of *P. vannamei* juveniles from Thailand into a land-based facility with recirculation filtration system (OIE 2020a). Following detection all animals were disposed of and the farm was disinfected.

Outside Asia, AHPND has been reported in Egypt (Megahed 2018) and Central and South America, although countries were not specified in the publications (Cuéllar-Anjel & Brock 2018; Han et al. 2016; Kanrar & Dhar 2018a; Restrepo et al. 2016). Country specific reports are available for Mexico (Nunan et al. 2014), Peru (Vicente et al. 2020) and Costa Rica (Peña-Navarro et al. 2020). An isolated event of the disease occurred in Texas, United States of America, but was controlled and eradicated (Dhar et al. 2019; OIE 2017c).

Prevalence

AHPND prevalence has been reported to be up to 90% in farms in regions where AHPND is present (FAO 2017; OIE 2021b; Tran, Fitzsimmons & Lightner 2014). A study in Vietnam reported prevalence of 78.5% (984/1254) in Mekong Delta farms during 2012–2013 (Boonyawiat 2017; FAO 2017). In Thailand, decreasing monthly prevalence on farms of 27%, 15% and 9% were reported during second half-2014, all-2015 and first 4 months-2016, respectively (n=around 31700 prawn samples annually) (FAO 2017; Songsangjinda 2017). However, a later survey reported a prevalence of 24% following a sampling of 150 Thai ponds (Shinn & Griffiths 2017). In Malaysia, 50% (197/394 prawns), 26% (151/584 prawns) and 34% (212/661 prawns) of farmed *P. vannamei* samples taken during 2011, 2012 and 2013, respectively were positive for AHPND (Chu et al. 2016). By 2014 and 2015 the prevalence in prawn samples had dropped to 12% (199/1,586) and 4% (50/1346), respectively (Chu et al. 2016). The prevalence in *P. monodon* samples during the same period was 10% (5/50) and 5% (4/74), respectively (Chu et al. 2016). A survey over a period of 4 years in 381 farms in India show no presence of Vp AHPND (Navaneeth et al. 2019). In Costa Rica, pVA1 and PirA and PirB genes were

detected in about 33% of prawn samples from a survey carried out between 2017 and 2018 in prawn farms (Peña-Navarro et al. 2020). Vp AHPND DNA was detected in 2% (1/60) of frozen *P. vannamei* imported from Vietnam to the Republic of Korea and collected from retail markets (Han et al. 2019b).

No reports of Vp AHPND prevalence in wild prawn populations were found.

Mortalities

Mortalities of 50–100% have been reported in farmed prawns in South-East Asia and Mexico, however virulence is reported to be variable between isolates of Vp AHPND (Akazawa et al. 2014; de la Peña et al. 2015; FAO 2013; Joshi et al. 2014a; Joshi et al. 2014b; Nunan et al. 2014; Soto-Rodriguez et al. 2015; Tran et al. 2013a). Mortalities due to AHPND commonly occur within 30–35 days after stocking (OIE 2021b). Although, mortalities can occur as early as 10 days (Joshi et al. 2014b; Nunan et al. 2014; Soto-Rodriguez et al. 2015; Tran et al. 2013b) and as late as 56–100 days after pond stocking (de la Peña et al. 2015; Vicente et al. 2020).

Transmission

Natural horizontal transmission occurs through ingestion of infected tissues and by ingestion of the agent in water (Tran, Fitzsimmons & Lightner 2014). Experimentally, AHPND has been transmitted *per os*, by immersion and reverse gavage (Nunan et al. 2014; Tran et al. 2013a; Tran et al. 2013b). Vp AHPND has been detected in faecal samples from AHPND-infected prawns, suggesting faecal contamination of water may also be a transmission route (OIE 2021b).

Transmission via live feed, such clams, oysters, polychaetes, and fresh squid meat, has been suggested following the detection of Vp AHPND in live feed samples (Thitamadee et al. 2016). A study from Vietnam showed that 3–5% of live polychaete samples tested positive to Vp AHPND and this rose to 90% in the Phan Rang region when there was a change in the environment (Tran 2018). It was speculated that specific pathogen free prawns became Vp AHPND-positive after being fed live polychaetes and clams (FAO 2017; Thitamadee et al. 2016).

The ubiquitous distribution of pVA1 and pVA1-like plasmids, and the suggestion that pVA1 and pVA1-like plasmids may be self-transmissible as they harbor a cluster of conjugative transfer genes, have led some authors to propose that PirA and PirB genes may be frequently transferred among *V. parahaemolyticus* and other *Vibrio* species (Dong et al. 2017a; Dong et al. 2019a; Lee et al. 2015; Xiao et al. 2017). Later, horizontal transfer of pVA1-like plasmid between *Vibrio* species was demonstrated by the transfer of a pVA1-like plasmid from AHPND-causing *V. campbellii* strain to a non-AHPND *V. owensii* strain (Dong et al. 2019a). However, the PirA and PirB genes on the transferred pVA1-like plasmid were unstable and lost from the *V. owensii* strain after 2 sub-culture passages (Dong et al. 2019a). It is unknown if transfer of AHPND-causing plasmids to other bacteria could be a risk for the introduction of AHPND into new areas because the transferred plasmids appear unstable *in vitro* and the infectivity of the bacteria following this transfer has not been demonstrated. However, a report of the analysis of a Vp AHPND strain isolated from *P. vannamei* in the Republic of Korea found that the overall plasmid genome was most similar to one reported from *V. owensii* isolated from *P. vannamei* from China (Han et al. 2020), which might suggest that the plasmid transferred from *V. owensii* to *V. parahaemolyticus*.

Mechanism of spread

The introduction of Vp AHPND into new areas has been attributed to trade and movement of infected broodstock, postlarvae and live feed (Thitamadee et al. 2016). It is also suggested that the movement of fresh (never previously frozen) prawn tissue poses a risk of introduction of AHPND (Tran et al. 2013a).

Vp AHPND is thought to have been introduced into Mexico from Asia via movement of infected live prawns (FAO 2017). Conversely, it has also been proposed that AHPND-causing plasmids are distributed worldwide, genetically diverse between isolates and that there are no epidemiological links between Asian and Mexican AHPND outbreaks (Fu et al. 2017).

Live feeds are considered a major biosecurity threat for the introduction of AHPND. It has been suggested that Vp AHPND-positive live polychaetes imported from China may have been the major route for introduction of AHPND into Thailand (FAO 2017; Thitamadee et al. 2016).

Vp AHPND can also live independently, and once introduced to a new geographical region it can persist in marine environments, sediments and biofilms (Thitamadee et al. 2016).

Infectious dose

The minimum infectious dose of Vp AHPND required to cause AHPND in susceptible species by experimental challenge or natural infection is not known. However, *per os* bioassays showed that Vp AHPND can be successfully transmitted to *P. vannamei* (weighing approximately 3.0g) by being fed once with 10% body weight of pelleted feed soaked in a Vp AHPND culture at 1:1 ratio wt/vol. Experiments showed 100% mortality within 3 days (Nunan et al. 2014).

Bioassays have also shown that mortalities can be induced by immersion of prawns in Vp AHPND suspensions. *P. monodon* postlarvae (PL) 15, PL30 and PL45 immersed in seawater containing 2.7×10^7 Vp AHPND CFU/ml for 1.5 hours showed mortalities of about 65%, 81% and 2%, respectively, 20 hours post-infection (Deris et al. 2020). Mortalities approached 100% after 2–4 days following immersion of *P. vannamei* in a Vp AHPND suspension with initial bacterial density between 10^6–10^8 CFU/ml (Nunan et al. 2014; Tran et al. 2013b). After similar experiments, Soto-Rodriguez et al. (2015) reported that virulence of *V. parahaemolyticus* strains is dose dependent, and that below a density of 10^4 CFU/ml no mortalities are observed (Soto-Rodriguez et al. 2015).

13.2.3 Pathogenesis

Incidence of AHPND has been reported to increase during hot and dry seasons (OIE 2021b). Overfeeding, poor feed quality, poor seed quality, poor water quality, algal blooms or crashes are also factors that have been reported to lead to AHPND outbreaks (OIE 2021b).

Enterocytozoon hepatopenaei (EHP) has been reported to be a risk factor for AHPND. In a laboratory study EHP-infected prawns and healthy prawns were challenged with Vp AHPND. Higher mortalities (44–60%) were seen in the EHP-infected prawns compared to healthy prawns (0–18%) (Aranguren, Han & Tang 2017). White spot syndrome virus (WSSV) infection has also been reported to be a risk factor for Vp AHPND. Prawns initially exposed to WSSV, followed by Vp AHPND had faster and higher mortality than prawns exposed to Vp AHPND alone (Han et al. 2019a). It has also been hypothesized that bacteria in the genera *Delftia, Rhodococcus, Leifsonia* and *Shewanella* may act in an additive or synergistic way to increase Vp AHPND virulence (Flegel 2017; Sritunyalucksana 2017).

Like other pathogenic bacteria *V. parahaemolyticus* uses the quorum-sensing system (Gomez-Gil et al. 2014), likely to maintain an infective density above 10^4 CFU/ml and to express pathogenicity through the release of the PirA and PirB toxins which causes lesions and dysfunction of the hepatopancreas. As the infection progresses, the hepatopancreas is atrophied to such a degree that it causes the death of the organism (Soto-Rodriguez et al. 2015). The sloughed hepatopancreatic cells provide a substrate for bacterial growth and therefore secondary bacterial infections usually contribute to the destruction of the organ (Tran et al. 2013b).

Tissue tropism
Vp AHPND is reported to target gut-associated tissues and organs including hepatopancreas, stomach, midgut and hindgut (Lightner et al. 2012a; OIE 2021b).

Tissue titre
Few studies have examined the titre of Vp AHPND in infected prawn tissues. Megahed (2018) reported a Vp AHPND bacterial density of $5.98 \times 10^6 \pm 6.51 \times 10^4$ CFU/g in the hepatopancreas of AHPND-affected *P. semisulcatus* from a prawn farm (Megahed 2018). The bacterial load in the hepatopancreas of *P. semisulcatus* challenged with Vp AHPND by intraperitoneal injection, was $5.79 \times 10^8 \pm 4.87 \times 10^8$ CFU/g 96 hours post-infection (Megahed 2018).

PCR analysis of hepatopancreas from frozen *P. vannamei* collected from retail markets in the Republic of Korea reported Vp AHPND DNA loads of about 2.5×10^3–3.4×10^3 total copies (Han et al. 2019b).

13.2.4 Diagnosis

Clinical signs
AHPND affected prawns typically show atrophied pale to white hepatopancreas together with an empty stomach and midgut (OIE 2021b). Atrophy of the hepatopancreas can reduce the size of the organ by 50% or more. Prawns with terminal phase of AHPND usually present black streaks or spots in the hepatopancreas due to melanin deposition from haemocyte activity (Tran et al. 2013b).

Pathology
The histopathology of AHPND presents two distinct phases, an acute phase and a terminal phase.

The acute phase shows a progressive degeneration of the hepatopancreas tubules, from proximal to distal, with sloughing and dysfunction of the tubular epithelial cells. The epithelial cells round up and detach from the affected tubules and become necrotic within the tubules or the gut lumen. A considerable number of bacteria may also be found in the stomach chamber with no significant bacterial colonization of the hepatopancreas tubule lumen (Lightner et al. 2012a; Tran et al. 2013b).

The terminal phase shows marked haemocytic infiltration and secondary bacterial infection in the hepatopancreas. Bacterial colonization is associated with necrotic and sloughed tubule epithelial cells (Lightner et al. 2012a; Tran et al. 2013b).

Testing
Vp AHPND can be isolated on standard media used for bacterial isolation (Lee et al. 2015; Soto-Rodriguez et al. 2015). Chapter 2.2.1 of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH 2022b) provides details of the methods currently available for targeted surveillance and

diagnosis of AHPND. Tests for targeted surveillance to declare freedom from infection with Vp AHPND are also recommended.

qPCR that targets PirA is described as the preferred testing method for declaring freedom from Vp AHPND (Han et al. 2015a; WOAH 2022b).

PCR (single step or nested) that detects the pVA1 plasmid (Dangtip et al. 2015; Flegel & Lo 2014) or the toxin genes PirA and PirB (Devadas et al. 2019; Han et al. 2015b; Sirikharin et al. 2015; Sritunyalucksana et al. 2015a; Tinwongger et al. 2014) are commonly used to diagnose AHPND. Histopathology, isothermal loop-mediated amplification, and a method using monoclonal antibodies specific to epitopes on PirB toxin are also available to detect AHPND (Arunrut et al. 2016; Koiwai et al. 2016; Wangman et al. 2019).

13.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments (OIE 2021b).

Vp AHPND has been shown to be susceptible to chloramphenicol and ofloxacin (Kongrueng et al. 2015; Lai et al. 2015). Vp AHPND have shown resistance to antibiotics including ampicillin, streptomycin, sulfamethoxazole, fosfomycin and bicozamycin (Lai et al. 2015). Other antibiotics such as kanamycin, tetracycline, nalidixic acid, trimethoprim and erythromycin are effective against some strains but not against others (Kongrueng et al. 2015; Lai et al. 2015). Vp AHPND strains with multiple antibiotic resistance genes have been reported (Devadas et al. 2018). The transfer of resistance plasmids and mobile genetic elements during recombination events is used by bacteria to achieve antibiotic resistance (Bennett 2008), therefore the frequent use of antibiotics in prawn aquaculture in some countries increases bacterial antibiotic resistance (Lai et al. 2015; Letchumanan et al. 2016).

13.2.6 control

Control measures for AHPND are primarily aimed at preventing the introduction of Vp AHPND into susceptible populations. Screening broodstock, postlarvae and feed sources by PCR to ensure absence of Vp AHPND before use is recommended (Thitamadee et al. 2016). Feed sources may also be frozen as there is evidence that Vp AHPND is inactivated by freeze-thaw cycles (Tran et al. 2013a).

Other general husbandry and disease control and management practices include the improvement of hatchery sanitary conditions, good pond preparation prior to stocking and good management of broodstock and farmed prawns (OIE 2021b; Thitamadee et al. 2016).

Microalgal-bacterial consortiums have been reported as a biocontrol strategy to provide growth inhibitory effects against Vp AHPND in farmed prawns (Chang et al. 2020). Some probiotics had been experimentally shown to be able to control AHPND-causing *V. parahaemolyticus* in the host's gastrointestinal tract and stimulate the prawn survival (Restrepo et al. 2021).

No AHPND-resistant domesticated stocks of penaeid prawns have been developed (OIE 2021b). However, genetic lines with expected small to moderate advances in the population by selection for the resistance indicators for AHPND have been reported (Campos-Montes et al. 2020). Immersion challenge of three Latin American prawn lines with 1×10^5 CFU/ml of Vp AHPND found that one of the lines displayed significantly higher survival rates (approximately 70%) compared to the other two lines and a specific pathogen free control group (Aranguren et al. 2020a). *E. carinicauda* from three

generations of selective breeding for resistance to Vp AHPND infection were injected with Vp AHPND at a dose of 10^7 CFU/ml. The 48 hour LD50 doses were determined to be $10^{6.0}$ CFU/ml for the first generation (G1), $10^{6.2}$ CFU/ml for the second generation (G2), and $10^{6.6}$ CFU/ml for the third generation (G3) (Ge et al. 2018). The survival rates of the same prawns at 144 hours post-infection were 26.67% in G1, 30% in G2 and 36.67% in G3 (Ge et al. 2018).

13.2.7 Impact of the disease

Economic losses due to AHPND have occurred in the prawn aquaculture industry in affected regions since 2009 (Anderson, Valderrama & Jory 2018; Chu et al. 2016; de la Peña et al. 2015; FAO 2013; Fegan 2017; Flegel 2012; Shinn & Griffiths 2017). The collective production losses for China, Malaysia, Mexico, Thailand, and Vietnam throughout 2010–2016 due to AHPND are estimated at 4.8 million metric tonnes, worth US$23.6 billion (Shinn et al. 2018b). A further loss of US$7 billion in feed sales and US$13.4 billion in export losses was estimated (Shinn et al. 2018b). It has also been reported that AHPND has caused ~60% drop to the prawn production in affected regions and global losses of US$43 billion to the prawn farming industry (Kumar et al. 2021).

No reports were found of the impact of infection with Vp AHPND on wild prawn populations.

13.2.8 Current biosecurity measures

Vp AHPND was not assessed in the Prawn IRA 2009 and there are no current biosecurity measures specific for Vp AHPND.

13.2.9 Conclusion

AHPND is present in exporting countries, is not present in Australia and is capable of causing adverse effects. In Australia, AHPND is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

It is noted that a member of the *Vibrio harveyi* clade and *Vibrio campbellii* have been isolated from prawns affected with hepatopancreatitis in Australia (Moody et al. 2019). The PirA and PirB toxin genes were identified in the pVA1 plasmid of the *V. harveyi* isolate, whilst in *V. campbellii* the toxin genes were not associated with the pVA1 plasmid (Moody et al. 2019). Based on the AHPND definition in the WOAH Code, the hazard 'Vp AHPND' is considered in the risk assessment. The pVA1 plasmid or *V. parahaemolyticus* are not considered on their own as they are both present in Australia, and not subject to control or eradication.

Given the ability of the pVA1 plasmid to transfer between *Vibrio* species (Dong et al. 2017a; Dong et al. 2019a; Lee et al. 2015; Xiao et al. 2017) it should be noted that the possibility exists that Vp AHPND could establish in Australia through natural means, that is through the transfer of endemic plasmids to endemic *V. parahaemolyticus*.

13.3 Risk assessment

Based on chapter 4 and the technical information about Vp AHPND presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if Vp AHPND meets Australia's ALOP are shown in Appendix F.

13.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for Vp AHPND were that:

- This risk review is generic and therefore the entry assessment assumes that Vp AHPND is present in all source countries.
- Vp AHPND infects various penaeid and caridean prawn species of marketable size that are exported to Australia.
- Prevalence of Vp AHPND in prawn farms may be up to 90%. No reports of the prevalence of Vp AHPND in wild prawns were found.
- Vp AHPND would be present in the whole body of infected prawns, but especially concentrated in the gut-associated tissues and organs including the hepatopancreas.
- The load of Vp AHPND in infected imported prawns is likely to be sufficient to cause infection in susceptible species.
- Post-harvest inspection may detect grossly abnormal prawns that are infected with Vp AHPND and remove them before export. Prawns with mild gross signs or no clinical signs would be unlikely to be detected.
- Vp AHPND in imported prawns is not expected to survive freezing, transport and storage and is unlikely to be infectious at the time of import.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of Vp AHPND in imported prawns was estimated to be **very low**.

13.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for Vp AHPND were that:

- Vp AHPND would be present in the whole body of infected prawns.
- The bacterial load of Vp AHPND in infected prawns is likely to be sufficient to cause infection in susceptible species if exposed.
- Due to its thermal sensitivity most Vp AHPND is not expected to persist and remain infectious in imported prawns (or associated wastes) at the point of exposure. However, any viable Vp AHPND which enter the marine environment would be capable of persisting as free-living organisms.
- Important aquaculture and wild-caught species in Australia that are susceptible to infection with Vp AHPND include *P. monodon*, *M. rosenbergii* and *P. japonicus*. Other Vp AHPND susceptible species such as *Artemia* are widespread in Australian waters. The impact of Vp AHPND on threatened native Australian species such as the critically endangered *Cherax tenuimanus* is unknown.
- Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustaceans would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a

farm has not implemented standards of entry-level biosecurity for intake water that would exclude Vp AHPND or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.

- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a small, yet significant volume of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Species susceptible to Vp AHPND may be present in research facilities and public aquaria.
- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley by recreational fishers. Prawn species susceptible to Vp AHPND are present in Australian waters and are likely to encounter imported prawns used as bait or berley. The host range for Vp AHPND is narrow compared to hazards such as WSSV or yellow head virus genotype 1, therefore the likelihood is less than for other hazards.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to Vp AHPND in imported prawns was estimated to be:

- Farmed crustaceans—**Extremely low**.
- Hatchery crustaceans—**Extremely low**.
- Wild crustaceans—**Low**.

13.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to Vp AHPND in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Extremely low**.
- Hatchery crustaceans—**Extremely low**.
- Wild crustaceans—**Very low**.

13.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)

The key points considered relevant when determining the partial likelihood of establishment and spread for Vp AHPND were that:

- Vp AHPND can be transmitted by ingestion of infected tissues and via water, it can also survive as a free-living organism in the aquatic environment.
- It is expected that susceptible species feeding on Vp AHPND-infected prawns would receive an infectious dose.

- It is unknown if prawns that survive infection with Vp AHPND can remain infectious.
- *P. monodon* which is the main prawn species farmed in Australia and a target species for fisheries, is susceptible to Vp AHPND infection.
- Other Vp AHPND susceptible species are found in Australian waters and include species important for the wild-caught fishery industry, such as *M. rosenbergii*. Also found are *Artemia*, polychaetes and bivalves which may be vectors for Vp AHPND.
- The likelihood of Vp AHPND establishment, following a given quantity of Vp AHPND entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.
- If one or more index cases of Vp AHPND were to occur in the wild, establishment and spread would be less likely than on a farm because infected wild animals (particularly those clinically affected) are likely to be prey for non-susceptible animals and the densities of susceptible and infected animals are much lower which reduces likelihood of transmission. However, because Vp AHPND can survive in the environment as a free-living bacterium, it could persist in an infectious form until susceptible hosts were to encounter it. Because the host range is limited for Vp AHPND, it would likely take some time to spread to its natural geographic limits, however it would be more likely than for those hazards which cannot survive outside of a host for long periods.
- If Vp AHPND were to establish in the wild, especially in waters around prawn farms, it could spread to farms through water intake due to Vp AHPND being able to survive as a free-living organism. Polychaetes and bivalves harvested from the local area and therefore harbouring Vp AHPND, could be deliberately introduced into the farms as feed for broodstock. Additionally, in the absence of effective biosecurity measures, wild infected prawns and polychaetes may be transferred into the farms through the inlet water channels. There are no known non-prawn crustacean species susceptible to Vp AHPND which are capable of surviving and moving outside of the water column, for example crabs. Therefore, infected wild crustaceans would only be able to enter farms through the water inlet channels, and not via movement across land.
- If Vp AHPND were to establish on a farm it could spread to neighbouring farms and wild populations through effluent water. This spread would be moderated by dilution effects and implementation of biosecurity measures on the farm should an incursion of Vp AHPND be suspected and response measures initiated. However, Vp AHPND is effectively transmitted through water and can persist in the environment as a free-living organism and farms which share a common water source with an infected population are likely to be exposed to Vp AHPND.
- The likelihood of Vp AHPND spread from farms to wild populations or neighbouring farms via escaped prawns is reduced due to the systems in place on farms to prevent discharge of live animals, however Vp AHPND could spread this way.
- Spread of Vp AHPND from hatchery crustaceans to the wild is unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities. However, given Vp AHPND survives as a free-living organism, if there were ineffective water treatment at these facilities it may aid in the spread of Vp AHPND from hatchery to the wild more so than for other hazards which can only survive for short periods without a host.
- Spread of Vp AHPND from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae as prawn species cultured in Australia are susceptible to Vp AHPND.

Vp AHPND is likely to be effectively transferred between hatchery and farm because postlarvae may not show clinical signs of infection until after transfer.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of Vp AHPND in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**High**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of Vp AHPND were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- Australia's main farmed prawn species are susceptible to Vp AHPND. There is high morbidity and mortality associated with infection.
- Vp AHPND would not be expected to impact wild fisheries in Australia. There are no reports of Vp AHPND in wild prawns and no reports of declines in catch rates or associated mortalities.
- Significant impacts have been reported in other countries where AHPND has established. Based on the impacts in Asia from Vp AHPND infection, Vp AHPND establishment and spread in Australia would be expected to cause significant production impacts at the national level.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- Susceptible species are distributed in Australian waters, however Vp AHPND has not been detected in wild populations elsewhere in the world.
- There are no reports about serious effects of Vp AHPND infection in wild prawn populations overseas. Whilst the environmental effects of Vp AHPND establishment and spread are unknown, they are expected to be limited given the apparent lack of impact in regions where Vp AHPND is endemic.
- The direct impact of Vp AHPND establishment and spread on the environment is not expected to be discernible at any level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- AHPND is listed as a notifiable disease by the WOAH and is included on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). State and territory governments would be expected to report on the agent.
- If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely.
- If Vp AHPND were to establish in wild populations in Australia, eradication would be near impossible as the agent is able to persist in marine environments, sediments and biofilms.

- If Vp AHPND spread into the wild, zoning and movement restrictions would be extensive as it would need to include penaeid and caridean prawns, *Artemia* and polychaetes and may not be successful given the ability of Vp AHPND to survive as a free-living organism.
- If a movement regulated area were put in place for an outbreak of Vp AHPND, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.
- Eradication of Vp AHPND would be expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Other industries such as seafood suppliers, commercial wild catch fisheries and the bait industry may be affected due to the host range of Vp AHPND including polychaetes, prawns and clams.
- Effects can also occur in all potential VpAHPND susceptible species and vectors which may be indirectly affected by movement regulated areas.
- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted, or decreased, feed companies would be impacted by reduced feed purchases.
- Vp AHPND infected prawns would likely show gross signs which may affect their marketability.
- Vp AHPND establishment and spread would likely have a minor impact at the national level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Vp AHPND is a WOAH-listed disease. Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. Vp AHPND establishment and spread may result in loss of some export markets due to importing country biosecurity requirements.
- If Vp AHPND were to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product.
- The impacts of Vp AHPND establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- Vp AHPND host range includes *C. quadricarinatus,* although infection of this species does not cause clinical signs.
- It is unknown if *Cherax tenuimanus* is susceptible to infection with Vp AHPND. However, *C. tenuimanus* is listed as critically endangered, and if Vp AHPND were to cause disease in *C. tenuimanus* it could have a significant impact on the survival of this already endangered species.
- In light of the uncertainty surrounding the susceptibility of *C. tenuimanus* to infection with Vp AHPND, a conservative approach has been adopted when considering the susceptibility of native species, particularly those that are endangered or threatened, and it is assumed they are susceptible.

- The impact of Vp AHPND establishment and spread on the biodiversity of the environment is considered to be minor at the national level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Prawns that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of Vp AHPND which may impact on social amenity.
- The social impacts of Vp AHPND establishment and spread are expected to be minor at the local level.

Table 19 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of Vp AHPND. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 19 Overall impact of establishment and spread of Vp AHPND for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Significant	F
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	National	Minor	E
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	National	E
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	B

Conclusion
The overall impact of establishment and spread of Vp AHPND was estimated to be **high**.

Determination of likely consequences of the outbreak scenario
The likely consequences of the outbreak scenario for Vp AHPND in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**High**.
- Hatchery crustaceans—**Moderate**.
- Wild crustaceans—**High**.

13.3.5 Determination of partial annual risk

The partial annual risk of Vp AHPND entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Negligible**.
- Wild crustaceans—**Low**.

13.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with Vp AHPND in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **low**.

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

13.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for Vp AHPND in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 13.4.1, 13.4.2 and 13.4.3 present the factors considered and the conclusions reached.

13.4.1 Head and shell removal

When determining if head and shell removal would reduce the overall risk of Vp AHPND to meet Australia's ALOP, the key points considered were:

- In the unrestricted risk assessment it was considered that freezing played a significant role in managing biosecurity risks of Vp AHPND in imported prawns by reducing the likelihood of entry (refer section 13.3.1 Entry assessment), however Australia's ALOP was not achieved. It is considered that head and shell removal of frozen prawns, further reduces the likelihood of entry of Vp AHPND. This is because head and shell removal significantly reduces the amount of Vp AHPND present in the imported prawn.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Head and shell removal is expected to further reduce the likelihood of this occurring because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed for aquatic animals held in research or public aquaria.
- Whole uncooked prawns are preferred over other forms (including uncooked and shelled) of prawns for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.
- The potential for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels remains, however when that likelihood is combined with the reduction in viable agent the overall likelihood is still considered negligible.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal, applied was determined to be **very low**.

13.4.2 Cooking

When determining if cooking would reduce the overall risk of Vp AHPND to meet Australia's ALOP, the key points considered were:

- *V. parahaemolyticus* in seafood is sensitive to heating. No reported investigations specific to the stability of Vp AHPND to heat treatments were found. Crustacean products that have been subjected to heat treatment sufficient to attain a core temperature of at least 100°C for at least 1 minute (or any time/temperature equivalent that has been demonstrated to inactivate Vp AHPND) are considered safe according to the WOAH code (WOAH 2022a).
- Given the available data regarding the effect of heating on Vp AHPND infectivity, it is assumed that cooking may reduce, but not completely inactivate Vp AHPND. Some infectious Vp AHPND may remain. Therefore, cooking to attain a core temperature of at least 65°C is expected to reduce the likelihood of entry, but not completely remove it.
- In the unrestricted risk assessment it was considered that freezing played a significant role in managing biosecurity risks of Vp AHPND in imported prawns by reducing the likelihood of entry (refer section 13.3.1 Entry assessment), however Australia's ALOP was not achieved with freezing on its own.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **very low**.

13.4.3 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of Vp AHPND to meet Australia's ALOP, the key points considered were:

- Value-added products are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). The likelihood of entry of Vp AHPND is expected to be the same as for head and shell removal. This includes a reduction in entry likelihood due to freezing and because it is not expected that the

processing will further reduce the amount of viable Vp AHPND in the product more than head and shell removal does.

- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

Conclusion

Based on this information, the overall restricted risk of imported uncooked frozen prawns which have been processed into a value-added product, was determined to be **negligible**.

14 White spot syndrome virus risk review

14.1 Background

White spot syndrome virus (WSSV) is the aetiological agent of white spot disease (WSD) (OIE 2021k). WSSV is classified by the International Committee on Taxonomy of Viruses (ICTV) as a member of the family *Nimaviridae* (Lo et al. 2011). A wide range of decapod crustaceans, including penaeid and caridean prawns, crayfish and lobsters are susceptible to infection with WSSV (OIE 2021k; Pradeep et al. 2012; Stentiford, Bonami & Alday-Sanz 2009).

Serious losses in farmed prawns due to WSSV were first reported from Asia in 1991 (Chou et al. 1995; Inouye et al. 1994; Momoyama et al. 1994; Takahashi et al. 1994). WSSV has since spread throughout most prawn culture areas of the Indo-Pacific, the Americas and the Middle East (Escobedo-Bonilla et al. 2008). WSSV is still considered the most serious threat to *Penaeus monodon* and *Penaeus vannamei* farmers in Asia (Stentiford, Bonami & Alday-Sanz 2009; Thitamadee et al. 2016).

Infection with WSSV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). A WSD outbreak occurred in farmed prawns in Australia in late-2016 but the outbreak is limited to South-East Queensland where it is under an official control program (Department of Agriculture and Fisheries 2019). Another outbreak was reported in August 2022 into northern New South Wales (NSW). This incident was quickly contained and eradicated. In early-2023, WSD was again detected at three prawn farms on the NSW north coast. The affected farms were issued with biosecurity direction by NSW Department of Primary Industries (DPI) to carry out destruction of affected animals and decontamination of the premises. Surveillance is being conducted to determine if the virus has been eradicated from the farms and to determine if the virus is present in the immediate environment in the vicinity of the farms. At the time of preparing this report, there is no indication that the virus has spread beyond the affected farms. NSW DPI' authorised officers have confirmed the biosecurity containment measures continue to be maintained to prevent further spread.

14.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of WSSV is warranted.

14.2.1 Agent properties

WSSV is an enveloped, circular, double-stranded DNA virus (Chou et al. 1995; Wang et al. 1995; Wongteerasupaya et al. 1995b) that is classified by the ICTV as a member of the genus *Whispovirus*, in the family *Nimaviridae* (Lo et al. 2011). The virions are ovoid or ellipsoid to bacilliform in shape measuring 120–150nm in diameter and 270–290nm in length, sometimes with a flagella-like appendage at one end (Lo et al. 2011).

Before WSSV was recognised as the generic virus name, it was given many different names including:

- Chinese baculovirus
- hypodermal and hemotopoietic necrosis baculovirus
- *Penaeus monodon* non-occluded baculovirus II-III
- Penaeid rod-shaped DNA virus
- rod-shaped nuclear virus of *Penaeus japonicus*
- systemic ectodermal and mesodermal baculovirus
- white spot bacilliform virus
- white spot baculovirus (Lightner 2011; Lo et al. 2011).

Various geographical isolates of WSSV with genotypic variations have been identified but all are classified as a single species of WSSV (Lo et al. 2011; Marks et al. 2004; Oakey & Smith 2018; Wongteerasupaya et al. 2003).

Several studies have investigated the stability of WSSV under various conditions. WSSV can remain infectious following exposure to freezing temperatures (–20°C to –70°C) for prolonged periods (Aranguren et al. 2020b; John et al. 2010; Lightner et al. 1997b; Nunan, Poulos & Lightner 1998; Reddy, Jeyasekaran & Jeya 2010; Wang et al. 1997; Wang et al. 1999a).

Experimental trials conducted using infectious material obtained from prawn carcasses has revealed that WSSV can retain infectivity for 6 days at 25.5°C to 28.8°C (Wang et al. 2002). In comparison, Prior et al. (2002) found prawn heads from WSSV-infected animals retained infectivity for at least 14 days, and that prawn tails can remain infectious for at least 28 days at 27°C. Additionally, WSSV was found to remain infectious in seawater for at least 30 days at 30°C, 120 days at 15°C (Momoyama et al. 1998) and for 3–4 days in ponds (Nakano et al. 1998). A further study found WSSV was viable and infectious in seawater of 27ppt, pH 7.5 at 30°C to 32°C (with initial viral load of 1000 virions/ml) for up to 12 days (Kumar et al. 2013).

There are variable reports about the effect of heat treatment on WSSV ability to remain infectious. Heat treatment has been shown to inactivate WSSV suspended in sterile water at 55°C for 90 mins and 70°C for 5 mins (Chang, Chen & Wang 1998b). WSSV has also been shown to be inactivated at 50°C for 60 mins, 60°C for 1min, 70°C for 0.2 mins in tissue homogenates (Nakano et al. 1998) and at 60°C for 20 mins for homogenised viral preparations (Balasubramanian et al. 2006). Conversely, experiments conducted on frozen WSSV-positive prawns demonstrated that WSSV DNA could only be destroyed by cooking the prawns at 100°C for 15 mins and quickly freezing at –40°C (Reddy, Jeyasekaran & Shakila 2011). Storing the prawns at 4°C, –20°C, –40°C, cooking at 100°C for 30 mins, or canning did not destroy the WSSV DNA (Reddy, Jeyasekaran & Shakila 2011). Further experiments have described how prawns inoculated with material from WSSV-infected prawns cooked at 100°C for 5, 10, 15, 20, 25 or 30 mins suffered 100% mortality 123 hours post-infection for all treatment groups (Reddy, Jeyasekaran & Jeya 2011). However, there were significant limitations to that study including that the PCR method used was not the method described by the WOAH, the moribund prawns did not have typical signs of WSD and the amplicon from the nested PCR was not sequenced to confirm it was WSSV. A later study found that prawns which were fed tissue from WSSV-positive prawns which had been boiled at 100°C for 1, 3, 5, 10 or 30 mins did not develop WSD as confirmed by histology, nested PCR and qPCR (Aranguren et al. 2020b). The department commissioned the University of Arizona in the United States of America to conduct independent research to determine

the minimum core temperature required to inactivate WSSV and yellow head virus genotype 1. WSSV-infected prawns were cooked to reach specific core temperatures ranging from 60°C to 95°C. They were then fed as minced cooked tissue to specific pathogen free *P. vannamei*. WSSV-infected prawns cooked to a core temperature of 60°C, 70°C, 75°C, 85°C, and 95°C were not capable of causing WSSV-infection in naive prawns (Aquaculture Pathology Laboratory & Department of Agriculture 2022a).

Treatment with ultraviolet light, ozone, low and high pH, sodium hypochlorite, povidone iodine, benzalkonium chloride and formalin have been shown to deactivate WSSV to varying degrees (Balasubramanian et al. 2006; Chang, Chen & Wang 1998b; Nakano et al. 1998; Oseko et al. 2006).

Irradiation (from a Cobalt-60 source) applied at a dose rate of 0.8 kilogray (kGy)/h for 12–36 hours has been shown to decrease the infectivity of a 30ml virus preparation of WSSV, but can only partly decrease the infectivity of WSSV in infected whole prawns (Liu et al. 2004). The optimum dose of irradiation for WSSV inactivation was reported as 10–15kGy (Motamedi-Sedeh, Afsharnasab & Heidarieh 2016; Motamedi-Sedeh et al. 2017). Values were calculated according to a dose/survival curve by using electron beam irradiation at different doses applied to WSSV preparations that were injected intramuscularly into experimental prawn populations (Motamedi-Sedeh, Afsharnasab & Heidarieh 2016; Motamedi-Sedeh et al. 2017). However, it is noted in these studies that suspensions of free virus were exposed to gamma irradiation rather than virus contained in tissue and whether the WSSV was still able to cause infection was not determined.

14.2.2 Epidemiology

Host range

A wide range of decapod crustaceans, including prawns, crabs, crayfish, and lobsters from marine, brackish and freshwater sources are considered susceptible to WSSV infection through both natural and experimental exposure (OIE 2021k; Pradeep et al. 2012; Stentiford, Bonami & Alday-Sanz 2009). However, variation in disease severity occurs across the Crustacea (Verbruggen et al. 2016). For example, although WSSV causes severe mortality in farmed prawns, it is not necessarily fatal to other hosts. Infection with WSSV results in little pathology and low mortality rates in the shore crab (*Carcinus maenas*), which has been confirmed as susceptible to WSSV infection (Bateman et al. 2012).

WSSV has been reported to be able to naturally infect and replicate in the foregut epithelium of the polychaete *Dendronereis* spp., which constitutes the first evidence of WSSV infection and replication in a non-crustacean host (Desrina et al. 2013).

All life stages of penaeid prawns, from postlarvae to adults, are susceptible to infection with WSSV (Lightner et al. 1998; Stentiford, Bonami & Alday-Sanz 2009).

Geographical distribution

Outbreaks of WSD were first reported in prawns in China, Taiwan and Japan between 1991 and 1993 (Chou et al. 1995; Escobedo-Bonilla et al. 2008; Inouye et al. 1994; Momoyama et al. 1994; Takahashi et al. 1994). Infection with WSSV was then detected throughout Asia (Flegel 2006), the Americas (Escobedo-Bonilla 2016; Lightner 2011), the Mediterranean (Stentiford & Lightner 2011), the Middle East (Yap 2001) and Africa (Le Groumellec 2012). In Australia, WSD is limited to an area in South-East Queensland and is under an official control program (Department of Agriculture and Fisheries 2019).

WSSV was detected in August 2022 in prawn in a biosecure facility in northern New South Wales. All affected animals were destroyed and the single affected farm has been decontaminated with the aim of eradicating the virus.

Prevalence

Prevalence of WSSV of up to 100% have been reported in farmed prawn populations (OIE 2021k). The Prawn IRA 2009 reported on a range of epidemiological studies in multiple penaeid species and multiple countries that showed WSSV prevalence in farmed prawns ranged from 48–90% (Biosecurity Australia 2009). Prevalence data from prawn farms since then show WSSV incidence ranged from 12–66% (Hossain et al. 2015; Soltani et al. 2018; Stentiford & Lightner 2011; Thamizhvanan et al. 2019). For example, WSSV was found to be present in 136/335 prawn samples (40% prevalence) collected from prawn farms, hatcheries and wild catching centres located along the east and west coast of India (John et al. 2010). A survey of farmed *Penaeus indicus* in Bushehr province in the Islamic Republic of Iran found 91% were PCR positive for WSSV (182/200) (Afsharnasab et al. 2007). In a survey of WSSV prevalence in 220 *P. vannamei* farms in Taiwan between 2004–2006, farms producing juveniles had the highest prevalence (38%; 19/50), followed by farms of sub-adults (34%; 17/50), adult farms (20%; 10/50), postlarvae farms (20%; 10/50) and broodstock farms (5%; 1/20) (Cheng et al. 2013).

The prevalence of WSSV among 820 samples of wild crustacean species collected from 59 sampling sites in the Bohai Sea in China were 17.4%, 12.2% and 7.9% in 2016, 2017 and 2018, respectively (Xu et al. 2021b). In this same study, the percentage of sampling sites testing positive for WSSV was 76.7%, 55.0% and 43.8% in 2016, 2017 and 2018, respectively (Xu et al. 2021b). Also, 11 out of the 19 sampled species of dominant wild crustaceans in the Bohai Sea were identified to be WSSV positive, including *Alpheus distinguendus*, which had the highest percentage of WSSV-positive results (21.93%, 25/114) (Xu et al. 2021b). WSSV was detected in 3/6 (50%) sites that wild *P. monodon* were caught from across the Philippines (Orosco & Lluisma 2017). In India, 26% (39/151) of wild *P. monodon* collected from across the coast of Andaman and Nicobar Islands were positive for WSSV (Saravanan et al. 2017). Eleven wild decapod crustacean species were collected between 2012–2015 from the Mississippi Sound, United States of America and tested for the presence and quantity of WSSV. Prevalence ranged from 5–39% across the species (Muhammad et al. 2020). During delimitation surveillance conducted for wild crustaceans from January to March 2017 in waters around the prawn farms affected by WSD in South-East Queensland, WSSV prevalence at each site for 11 sampling sites was estimated to range from 2–28% (Hood et al. 2019). In a surveillance survey conducted in March 2020 at 11 sampling sites in northern Moreton Bay, Queensland (which previously tested positive for WSD in March 2018), low levels of WSSV were again detected in several wild crabs and prawns (sample numbers not provided) (NACA, OIE-RRAP & FAO 2020a).

Mortalities

WSSV infections in farmed prawn populations frequently result in cumulative mortalities of up to 100% within 3–10 days of the onset of clinical signs (Chou et al. 1995; Inouye et al. 1994; Lightner 1996a; Wongteerasupaya et al. 1995b). In Australia, mortalities of up to 90% within 8 days were reported from ponds in prawn farms on the Logan River during the outbreak of WSD (Commonwealth of Australia 2017).

Infection has also been observed to persist throughout the crop cycle, with only occasional mortality (Tsai et al. 1999), and good aquaculture harvests can be obtained within 1.5 years of the introduction of WSSV when recommended management techniques are adopted (Flegel 1997b).

WSSV has been detected in wild prawn populations but there are no reports of declines in catch rates or associated mortalities (Biosecurity Australia 2009; Saravanan et al. 2017). The absence of an observable effect on wild prawn populations may be due to lower stress levels in wild prawns, lower levels of infection or a lower ability of methods used to detect significant impacts to wild crustacean populations (Shields 2012; Stentiford 2012).

Transmission

Natural transmission of WSSV can be horizontal by ingestion of infected tissue (Chang et al. 1996; Wang et al. 1999b) or by waterborne transmission through shedding of the virus into the water or ingestion of the pathogen from WSSV-contaminated water (Chou et al. 1995; Kanchanaphum et al. 1998; Wang et al. 1997). Infection *per os* has been shown to be a more effective inoculation route than immersion in viral extract or cohabitation (Perez, Volckaert & Calderón 2005; Soto & Lotz 2001). Water can be contaminated with faecal pellets from WSSV-positive animals (Rajan et al. 2000). Dead and moribund prawns can be a source of WSSV transmission (Lo & Kou 1998; Soto, Shervette & Lotz 2001). Experimentally, WSSV can also be transmitted by injection of infected inoculum (Balasubramanian et al. 2006; Momoyama et al. 1998; Nunan, Poulos & Lightner 1998; Takahashi et al. 1994).

WSSV DNA has been detected in reproductive organs by PCR analysis (Lo et al. 1997a) and eggs, nauplii and postlarvae spawned from WSSV-positive broodstock became infected with WSSV (Hsu et al. 1999; Peng et al. 2001; Tsai et al. 1999). It is unclear whether transovarial transmission of WSSV takes place, or whether the spread of virus from broodstock to progeny occurs via contamination of the external surface of the egg or via release of the virus during spawning which is subsequently ingested by larval stages (Chang, Chen & Wang 1998a; Lo & Kou 1998). It has also been suggested that oocytes that contain the virus do not develop to mature eggs, as WSSV particles have been reported to be absent in mature eggs (Lo et al. 1997a).

A wide range of decapod crustaceans, including crabs, are known to be reservoir hosts of WSSV (Escobedo-Bonilla et al. 2008; Lo et al. 1996a; Pradeep et al. 2012; Stentiford, Bonami & Alday-Sanz 2009). In bioassays with *P. monodon* fed WSSV-infected crab and lobster tissues, clinical signs of WSD and mortality occurred in the prawns within 2–4 days (Rajendran et al. 1999). In cohabitation studies of *P. monodon* with WSSV-infected crabs, WSSV infection was confirmed in the prawns 2 days after exposure (by PCR) and cumulative mortalities reached 100% 8 days post-exposure (Kanchanaphum et al. 1998).

Some non-decapod species, such as copepods, rotifers, polychaetes, marine molluscs, sea slaters and aquatic insect larvae have been shown to mechanically transport WSSV (Escobedo-Bonilla et al. 2008; Flegel 2006; Haryadi et al. 2015; Lo et al. 1996a; Vijayan et al. 2005b; Wang et al. 2017a; Yan et al. 2004; Zhang et al. 2006). However, the polychaete *Dendronereis* spp. has been shown to be susceptible to WSSV, which constitutes the first evidence of WSSV infection and replication in a non-crustacean host (Desrina et al. 2013).

WSSV was detected by PCR in freshwater snails, *Melanoides tuberculate* and *Pomacea lineata*, in the Paraíba River, Brazil (Bandeira et al. 2019). Rotifers, commonly used as live feed in prawn aquaculture settings, collected from prawn ponds in China were found to be WSSV-positive by PCR and/or dot blot hybridization (Wang et al. 2017a; Yan et al. 2004). In an infection study, 40% of *Penaeus chinensis* postlarvae became WSSV-positive after feeding on WSSV-infected rotifers (Zhang et al. 2006), suggesting that rotifers may serve as vectors in WSSV transmission to prawns. Polychaetes such as *Marphysa gravelyi*, *Pereneis nuntia* and *Dendronereis* spp., were also shown to carry WSSV (Haryadi et al. 2015; Laoaroon et al. 2005; Vijayan et al. 2005b). In independent transmission studies, *P. monodon* and *P. vannamei* were infected with WSSV after feeding on WSSV-positive *Marphysa gravelyi* and *Dendronereis* spp, respectively (Haryadi et al. 2015; Vijayan et al. 2005b).

The detection of WSSV in prawn pond soil suggests that it too may serve as a reservoir of virus. Experimental studies showed that WSSV within prawn pond sediment remained viable and infectious up to 19 days with sun drying and up to 35 days under water-logged conditions (Kumar et al. 2013). WSSV DNA was also detected in soil samples collected from WSSV-infected prawn ponds and was found to persist when the soil was heated to 37°C for 5 days or stored for up to 10 months at room temperature in the dark (Natividad, Nomura & Matsumura 2008). However it is unknown if the virus in these samples was infectious.

Mechanism of spread

The introduction of WSSV into new areas has most often been attributed to the movement of live broodstock and postlarvae (Lightner et al. 1997b; Stentiford, Bonami & Alday-Sanz 2009; Stentiford & Lightner 2011; Takahashi et al. 1994). Transmission through ingestion of contaminated feed or prey is also an effective infection pathway (Pradeep et al. 2012).

There have been several studies showing WSSV remains viable and infectious in frozen prawns and WSSV-infected frozen prawns are suggested to have been responsible for the introduction of the virus into the United States of America (Bateman 2014; Durand, Tang & Lightner 2000; Hasson et al. 2006; Lightner et al. 1997a; Lightner et al. 1997b; Nunan, Poulos & Lightner 1998; Reddy, Jeyasekaran & Jeya 2010; Reville et al. 2005).

Prawn eating gulls or seabirds may also be a factor in the spread of WSSV by moving WSSV-infected dead or moribund prawns from prawn ponds and dropping them into unaffected waterways (Lightner et al. 1997b).

Other possible introduction pathways include the natural dispersion of either wild infected prawns or other susceptible crustaceans species (Galaviz-Silva et al. 2004). It was stated that WSSV genotypes isolated from farmed prawns in Saudi Arabia, Mozambique and Madagascar during 2010–2012 likely originated from wild populations in the Red Sea and Indian Ocean as other routes of exposure of farmed animals to WSSV were unlikely (Tang, Le Groumellec & Lightner 2013).

In Australia in 2000, WSSV DNA was detected in imported frozen raw prawns that were intended for human consumption but were fed to crustacean hatchery broodstock, including *Scylla serrata* and *P. monodon* (all exposed animals were destroyed). It is uncertain whether the incident resulted in a clinical disease, although WSSV was briefly detected in mud crabs near the hatchery outlet channel. Subsequent surveillance confirmed that the virus was no longer detectable (East et al. 2004).

Following an outbreak of WSD on a prawn farm on the Logan River in South-East Queensland, Australia in late-2016, WSSV is considered established in populations of wild crustaceans within the Queensland movement regulated area (MRA). The department's investigation into the cause of the outbreak focused on several possible entry pathways and to date, the origin of the outbreak has not been determined (Department of Agriculture and Water Resources 2017c). Although, others have expressed the view that retail prawns being used as bait were the most likely source of the infection (Diggles 2017). In August 2022, WSSV was detected for the first time outside of the MRA. Routine testing of prawn broodstock held in a biosecure facility in New South Wales was positive for WSSV and subsequent testing confirmed WSSV. All (400) animals within the facility were destroyed and the facility decontaminated. This incident was quickly contained and eradicated. In early-2023, WSD was again detected at three prawn farms on the NSW north coast. The farms were issued with a biosecurity direction by NSW DPI to carry out destruction of affected animals and decontamination of the premises. Surveillance is being conducted to determine if the virus has been eradicated from the farms and to determine if the virus is present in the immediate environment in the vicinity of the farms. At the time of preparing this report, there is no indication that the virus has spread beyond the affected premises.

Infectious dose

The minimum infectious dose of WSSV required to cause WSD in susceptible species by experimental challenge or natural infection is not known. Although, there are many reports of various methods and doses of WSSV which can elicit infection. It was reported that 2000 copies of WSSV genome resulted in a cumulative mortality greater than 80% 14 days post-infection when *P. vannamei* were exposed through oral gavage (Gitterle et al. 2006). Intramuscular injection of 1×10^4 WSSV copies into 5g *P. vannamei* resulted in 100% mortality within 4 days post-infection (Durand & Lightner 2002). Jeena et al. (2018) showed that injection of 5×10^6 WSSV genome copies into *P. vannamei* resulted in 100% mortality within 5 days post-infection. In an infection study of *P. monodon*, intramuscular injection (0.1ml) of WSSV stock at 2.62×10^6 genome copies/µl was sufficient to result in moribund prawns within 3 days post-infection (Gomathi, Otta & Shekhar 2015). The WSSV LD_{50} (amount of agent that causes an average 50% mortality of exposed animals) following intramuscular injection of *P. japonicus* is reported to be 950 genome copies of WSSV/g of prawn tissue (Wu et al. 2002). Similarly, the WSSV LD_{50} of experimentally infected (by intramuscular injection) *Macrobrachium nipponense* and *P. vannamei* was approximately 10^4 and 10^1 genome copies/g, respectively (Zhao et al. 2017). In a waterborne challenge experiment, a concentration of 1×10^5 WSSV copies/ml of sea water (6 *P. vannamei* submerged in a total volume of 5L) was sufficient to induce 100% mortality within 9 days (Durand & Lightner 2002). Thuong et al. (2016) showed that compared to intramuscular injection $10^{7.53}$ times more WSSV was needed to infect a prawn via gavage and $10^{8.03}$ times more WSSV was needed to infect a prawn via *per os* (Thuong et al. 2016).

14.2.3 Pathogenesis

Infection may be patent, latent or transitional. In patent infections, clinical signs (including mortality) are evident within 2–7 days. Latent infections may continue for extended periods; however, the transition period to patent infection is generally short, perhaps only lasting a few hours (Lo & Kou 1998). The transition to patent infection may be triggered by stressors, such as environmental stress during the monsoon season (Karunasagar, Otta & Karunasagar 1997), rainy periods in combination

with temperature and salinity fluctuations (Peinado-Guevara & Lopez-Meyer 2006), temperature changes (Korkut, Noonin & Söderhäll 2018), and spawning stress (Hsu et al. 1999).

Water temperature is one of the most important environmental factors that may impact a WSD outbreak (Korkut, Noonin & Söderhäll 2018). Various studies have demonstrated that temperatures lower than 16°C to 18°C or higher than 30°C can provide protection to crustacean species, including prawns, from development of disease associated with WSSV infection (Granja et al. 2006; Guan, Yu & Li 2003; Jiravanichpaisal, Söderhäll & Söderhäll 2004; Korkut, Noonin & Söderhäll 2018; Sonnenholzner & Calderón 2004; Vidal et al. 2001).

Tissue tropism

WSSV infects cells in a wide range of tissues such as the stomach, gills, cuticular epithelium, haemocytes, nervous tissue, antennal gland, lymphoid organ, muscle, midgut, hindgut and subcuticular connective tissue (Chang et al. 1996; Di Leonardo et al. 2005; Durand et al. 1996; Lo et al. 1997a; Wang et al. 1995; Wang et al. 1999b; Wongteerasupaya et al. 1995b).

Stomach and antennal gland are the main target organs for WSSV invasion (Liu et al. 2020b). After ingestion, the virus is thought to first infect epithelial cells of the digestive system and cells of the antennal gland. Following replication of the virus and host cell lysis, the virus circulates via the haemolymph to other body tissues (Huang et al. 2000; Liu et al. 2020b). Shedding via faeces is likely as inoculation of tank water with faecal pellets from WSSV-positive animals resulted in WSSV infection in previously uninfected prawns (Rajan et al. 2000).

Tissue titres

The WSSV load in infected prawns can be highly variable when calculated using qPCR. Many factors have been suggested that may influence variability, including host species, tissue tested and the route and stage of infection (Durand & Lightner 2002; Durand et al. 2003).

Prawns with WSD at the onset of mortality are reported to have very high WSSV loads, which are in the order of 10^9–10^{10} copies/g of tissue (Oidtmann & Stentiford 2011).

Experimentally infected moribund juveniles of *P. vannamei* and *Penaeus stylirostris* had mean viral loads of 1.6×10^9 and 3×10^{10} genome copies/µg of DNA, respectively (Durand & Lightner 2002). The WSSV load was quantified in experimentally infected prawn tissues (by injection) and the mean numbers of genome copies/µg of DNA in the tissues were 2.55×10^9 in haemolymph, 1.6×10^9 in pleopods, 1.2×10^9 in gills, 1.9×10^8 in muscle and 9×10^7 in the hepatopancreas (Durand & Lightner 2002).

Juvenile *P. vannamei* (mean weight 3g) in the acute phase of WSSV infection (experimentally infected *per os*) that were subjected to a simulated emergency harvest, contained 10^5– 10^9 WSSV copies/µg of total DNA and 10^5–10^{11} copies/g of host tissue with means in the range of 10^{10} copies/g for all tissues sampled (Durand et al. 2003). The study found that the prawn head had a higher WSSV load (2×10^{10} WSSV copies/g of tissue) than the tail (1.53×10^{10} WSSV copies/g of tissue). However, since the tail makes up about 58% of a *P. vannamei* prawn's total body weight and the head about 42%, the total virus load, on a per weight basis, was 49% in the head and 51% in the tail. If the prawns were harvested at 15g, this would translate to a viral load of 1.26×10^{11} WSSV copies in the head and 1.33×10^{11} WSSV copies in the tail. Within the tail, 55% of the WSSV viral load is in the tail shell, tail fan and pleopods and 45% is in the muscle, epidermis and connective tissue associated with the

hindgut and midgut. Of note, the WSSV loads in the tail shell, tail fan and pleopods (7.32×10^{10} WSSV copies/g of tissue) was about 4 times higher than that present in the tail muscle (1.86×10^{10} WSSV copies/g of tissue) (Durand et al. 2003).

A study on orally infected *P. vannamei* (weight 4g) kept at 26°C had a WSSV viral load of 6.3×10^6 genome copy number/µl of haemolymph 8 days post-exposure (Granja et al. 2006). *P. japonicus* experimentally infected by immersion had mean WSSV viral loads at 14 days post-exposure of 1.0×10^8 genome copies/µg of DNA in the gills and stomach and 1.0×10^7 genome copies/µg of DNA in the heart and lymphoid tissues (Ashikaga et al. 2009). Moribund *P. monodon* experimentally infected (by intramuscular injection) had WSSV copies of 3.0×10^7 in the gills, 2.7×10^7 in the gut, 1.5×10^7 in muscle, 3.3×10^6 in the haemolymph, 2.9×10^6 in the eyestalk, 2.5×10^6 in the pleopod and 2.4×10^6 in the hepatopancreas (Gomathi, Otta & Shekhar 2015). In another study, *P. vannamei* was challenged with WSSV (by injection) and the mean genome copy/µg of DNA in the tissues after 72 hours post-infection were 3.8×10^9 in sub-cuticular epithelium, 3.8×10^7 in pleopod and 3.81×10^6 in the gills (Jeena et al. 2018).

Qualitative analysis performed using *in situ* hybridisation techniques indicates that the number of WSSV-positive cells in wild-caught prawns was relatively low compared to the number of WSSV-positive cells in farmed and experimentally infected prawns (Lo et al. 1997b). The mean WSSV viral load found in pleopods of naturally infected, moribund *P. monodon* juveniles was 2.10×10^6 genome copies/µg DNA (Durand & Lightner 2002). Jang et al. (2009) reported a mean WSSV viral load of 1.5×10^4 genome copies/ng of DNA from pleopods of wild *P. chinensis* brooders collected in the Republic of Korea. The study found that 69.8% of the brooders recorded <10 WSSV copies/ng of DNA in the pleopods (Jang et al. 2009).

14.2.4 Diagnosis

Clinical signs

Prawns suffering from WSD may display various clinical signs including lethargy, reduced food consumption, pink to red discolouration of the body, the appearance of white spots (0.5–2.0mm in diameter) on the cuticle and high mortality (Chou et al. 1995; Durand et al. 1997; Lightner 1996a; Momoyama et al. 1994; Takahashi et al. 1994; Wang et al. 1995). The white spots are the result of calcified deposits by the cuticular epidermis (Lightner 1996b). Diagnosis of WSD should not be based on the presence of white spots on the cuticle as they are also produced during bacterial infections and under environmental stress factors and are often absent in WSSV-infected prawns (Flegel 2006; Hossain et al. 2015; OIE 2021k; Wang et al. 2000). In a study of *P. monodon* farmed along the coast of Bangladesh, it was found that 20% of prawns did not have external spots or characteristic symptoms of WSD, but they were positive for WSSV by PCR (Hossain et al. 2015).

WSSV-infected prawns have also been observed with a loosened attachment of the carapace with the underlying cuticular epithelium, delayed clotting of haemolymph and excessive fouling of the gills (OIE 2021k). The presence of clinical signs is variable, in some prawns the only sign noted is mortality.

Non-prawn crustaceans such as crabs, crayfish and lobsters generally do not show clinical signs of infection when infected with WSSV by natural exposure routes (OIE 2021k).

Pathology

Histological signs of WSSV infection include hypertrophied nuclei containing eosinophilic to basophilic inclusions and marginalised chromatin in infected tissues (Chang et al. 1996; Lightner 1996b; Lightner et al. 1997b; Wongteerasupaya et al. 1995b). This was most commonly seen in the cuticular epithelial cells and connective tissue cells (Lightner 1996b). Multifocal necrosis associated with pyknotic and karyorrhectic nuclei and tissue disorganisation become evident as the infection advances (Chang et al. 1996; Wang et al. 1997; Wongteerasupaya et al. 1995b).

Testing

The Prawn IRA 2009 provided an overview of various testing methodologies which may be used to diagnose WSSV (Biosecurity Australia 2009). Chapter 2.2.8 of the WOAH *Manual of diagnostic tests for aquatic animals* provides details of the methods currently available for targeted surveillance and diagnosis of WSSV. qPCR is the recommended test for targeted surveillance to declare freedom from infection with WSSV (WOAH 2022j).

Molecular methods such as PCR (Lo et al. 1997a; Lo et al. 1996a; Lo et al. 1996b; Maeda et al. 1997) and qPCR (Durand & Lightner 2002; Mendoza-Cano & Sánchez-Paz 2013; Sritunyalucksana et al. 2006b) are now commonly used for detection of WSSV.

In the past, histopathology and transmission electron microscopy (Chou et al. 1995; Takahashi et al. 1994; Wongteerasupaya et al. 1995b), along with dot blot hybridisation (Edgerton 2004) and *in situ* hybridisation (Chang et al. 1996; Durand et al. 1996; Lo et al. 1997a; Wongteerasupaya et al. 1996) were used to detect WSSV. Loop mediated isothermal amplification is another method for detection of WSSV (Mekata et al. 2009; Srisuvan et al. 2013). Various antibody based tests using either monoclonal or polyclonal antibodies against WSSV have been developed for WSSV detection (Anil, Shankar & Mohan 2002; Nadala & Loh 2000; Poulos et al. 2001; Sithigorngul et al. 2006; Yoganandhan et al. 2004; You et al. 2002) although they are no longer routinely used.

14.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments (OIE 2021k).

Immunostimulant treatments with beta-glucan, probiotics, macromolecules and vitamins have been suggested to improve resistance to WSSV infection (Chang et al. 2003; Chang et al. 1999; Chotigeat et al. 2004; Maeda et al. 1997; Rodríguez et al. 2007; Takahashi et al. 2000). Plant extracts were screened to identify those with anti-WSSV activity, with a few found with possible protective effects (Balasubramanian et al. 2008; Balasubramanian et al. 2006; Ghosh et al. 2014; Huang et al. 2020a; Sudheer, Philip & Singh 2011). However, these treatments have not been applied in the field.

14.2.6 Control

Control measures are primarily aimed at preventing the introduction of WSSV into susceptible populations and include stocking only specific pathogen free broodstock and postlarvae, screening and disinfecting water intake, constructing physical barriers to prevent access by wild crustaceans, preventing unrestricted movement of stock, avoiding cohabitation of different species, avoiding the use of fresh feed and stocking in the warm season (Flegel 1996; Limsuwan 1997; Lo & Kou 1998; Maeda et al. 1998; Peng et al. 2001; Vidal et al. 2001).

Where populations are already WSSV-infected, control measures focus on reducing the spread of the virus to neighbouring populations by treating and delaying the discharge of water from infected ponds, fallowing, optimising environmental conditions, reducing stress levels and generally improving husbandry methods (Flegel 1996; Limsuwan 1997; Lo & Kou 1998; Peng et al. 2001).

There is laboratory evidence that short-term protection against WSSV can be achieved through exposing prawns to 'vaccines' such as inactivated virus, recombinant viral proteins, viral DNA and double-stranded RNA (Motamedi-Sedeh et al. 2017; Namikoshi et al. 2004; Singh et al. 2005; Witteveldt et al. 2004).

Resistance breeding programs with the objective of producing *P. vannamei* families that tolerate and/or resist infections with WSSV have been in development since early 2000s with results showing an improvement of the resistance and commercial performance of the selected family lines (Campos-Montes et al. 2020; Cuéllar-Anjel et al. 2012; Gitterle et al. 2005; Huang et al. 2012; Trang et al. 2019). Whilst resistance breeding programs have shown success overseas, it is noted that *P. vannamei* are not present in Australia. Any resistance breeding programs in Australia would need to be established using prawn species present here, unless import requirements were to change in the future.

14.2.7 Impact of the disease

Globally, the economic losses due to WSD have been significant. Some have estimated total losses of at least US$8 billion since its emergence (Akazawa et al. 2014), whilst others have estimated the total losses to be US$15 billion (Lightner et al. 2012b). Annual losses have traditionally equated to approximately one tenth of global prawn production, which was estimated to equate to approximately US$1 billion of output lost per year due to WSD (Stentiford et al. 2012).

Production losses due to WSD in the Vietnamese Mekong Delta during 2015 were reported to range between US$ 11 million (Shinn et al. 2018a) to US$55.6 million (Hien et al. 2016). In 2016, annual prawn losses in Indonesia were estimated at US$191 million for WSD (Hastuti & Desrina. 2016). In 2016–17 in Australia, the prawn farming industry production losses due to WSD were estimated to be approximately AU$23.5 million with an additional AU$5–6 million lost in hatchery and breeding stocks (Commonwealth of Australia 2017).

Overseas, it has been reported that good prawn aquaculture harvests can be obtained within 1.5 years of the introduction of WSSV, despite the continued presence of the virus when recommended management techniques are adopted (Flegel 1997b). For example, in the Philippines successful harvests have been achieved consistently since 2010 (aside from emergency harvests in 2013 and 2014) by ensuring the use of clean postlarvae, good on-farm biosecurity, regular monitoring of water quality, *Vibrio* spp. and WSSV, and an understanding of each pond's carrying capacity (Merican 2018). In Australia, five farms on the Logan River commenced the production season in September 2020, following the detection of WSSV in prawns on two farms in the Logan River region in April 2020. There have been no reports of white spot disease to date. Biosecurity Queensland is working with all prawn farms on the Logan River to ensure on-farm biosecurity management is appropriate.

Whilst no reports were found describing significant declines in wild crustacean populations due to infection with WSSV, WSSV has been detected in wild crustacean populations (Diggles 2020; Hood et al. 2019; Muhammad et al. 2020; Orosco & Lluisma 2017; Saravanan et al. 2017).

14.2.8 Current biosecurity measures

The Prawn IRA 2009 determined the unrestricted risk associated with WSSV to be high and therefore biosecurity measures were necessary (Biosecurity Australia 2009).

Current biosecurity measures which manage risks for WSSV are:

- demonstration of source population freedom
- cooking
- highly processed prawn products (dumpling and dim sum type-products)
- breaded, battered or crumbed prawns
- head and shell removal (last segment and tail fan excluded) in combination with pre-export and on-arrival testing.

14.2.9 Conclusion

WSSV is present in exporting countries. In Australia, WSSV is limited to South-East Queensland and is under an official control program. WSSV can cause adverse effects. Based on the preceding information risk assessment is warranted.

14.3 Risk assessment

Based on chapter 4 and the technical information about WSSV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of WSSV meets Australia's ALOP are shown in Appendix F.

14.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for WSSV were that:

- This risk review is generic and therefore the entry assessment assumes that WSSV is present in all source countries.
- WSSV infects all penaeid and caridean prawn species of marketable size that are exported to Australia.
- Prevalence of WSSV in farmed and wild prawns is variable, but can be up to 100%.
- WSSV would be present in the whole body of infected prawns.
- The viral load of WSSV in infected prawns is likely to be sufficient to cause infection in susceptible species.
- Post-harvest inspection may detect grossly abnormal prawns that are WSSV-positive and remove them before export. Prawns with mild gross signs or which do not show clinical signs would be unlikely to be detected.
- WSSV in imported prawns is expected to survive freezing, storage and transport and remain infectious at the time of import.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of WSSV in imported prawns was estimated to be **high**.

14.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for WSSV were that:

- WSSV would be present in the whole body of infected prawns or in associated wastes that may enter the environment of the exposure groups.
- WSSV would be expected to be present in sufficient loads in infected prawns (or associated wastes) to cause infection in a susceptible species if exposed.
- WSSV in imported prawns (or associated wastes) is likely to persist and remain infectious in water at the point of exposure for an extended period.
- It is assumed that all decapod crustaceans in Australia, aquaculture and wild-caught species, would be susceptible to WSSV. This includes the main aquaculture species *P. monodon, P. merguiensis, Cherax destructor*, *C. quadricarinatus*, *C. cainii* and *C. tenuimanus*. Wild-caught crustacean species in Australia, including *P. monodon*, *P. merguiensis, M. rosenbergii* and high value rock lobster (*Panulirus cygnus*) are also susceptible to WSSV. The impact of WSSV on threatened native Australian crustacean species such as the critically endangered *Cherax tenuimanus* is unknown.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a farm has not implemented standards of entry-level biosecurity for intake water that would exclude WSSV or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.
- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Due to the broad host range of WSSV it is expected that any crustacean species present in research facilities and public aquaria would be susceptible to WSSV.
- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley and due to the wide host range of WSSV and the widespread presence of crustaceans in Australian waterways.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to WSSV in imported prawns was estimated to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Moderate**.

- Wild crustaceans—**High**.

14.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to WSSV in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Moderate**.
- Wild crustaceans—**High**.

14.3.4 Consequence assessment

Partial likelihood of establishment and spread

The key points considered relevant when determining the partial likelihood of establishment and spread for WSSV were that

- WSSV can be transmitted from broodstock to progeny, by ingestion of infected tissues, cohabitation and via water where it can remain infectious for an extended period.
- It is expected that susceptible species feeding on WSSV-infected prawns would receive an infectious dose.
- Prawns that survive WSSV infection can remain infectious and become sources of the virus.
- All decapod crustaceans farmed or wild-caught in Australia, including *P. monodon*, *P. merguiensis* and *P. japonicus,* are susceptible to WSSV infection and are widespread in Australian waters.
- WSSV susceptible species and vectors are present and widespread in Australia and include crabs, lobsters, polychaetes, copepods, rotifers, marine molluscs, snails, sea slaters and aquatic insect larvae.
- The likelihood of WSSV establishment, following a given quantity of WSSV entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.
- If one or more index cases of WSSV were to occur, establishment and spread in the directly exposed wild crustaceans is more likely compared to most other hazards. This is because although infected wild animals (particularly those clinically affected) are most likely to be prey for non-susceptible animals, in the case of WSSV not all decapod crustaceans susceptible to infection exhibit clinical signs (or mortality). For example, crabs and polychaetes have been demonstrated to be infected with WSSV but have not experienced significant pathology or high mortality rates. Therefore, it is more likely that WSSV will persist in a wild population and eventually spread to its natural geographic limits compared to other hazards. Additionally, the densities of susceptible animals in the wild are greater, given the very wide host range of WSSV, which provides more opportunities for transmission and therefore spread. Prawns that survive WSSV infection can remain infectious and become sources of the virus which could also aid in its spread.
- If WSSV were to establish in the wild, especially in waters around prawn farms, it may easily spread to farms due to being transmissible through water. In the absence of effective biosecurity measures, wild infected prawns, or vectors such as polychaetes, may be transferred into the

farms through the inlet water channels. There are known susceptible species of WSSV, such as all crab species, which can also enter farms through movement across short distances of land.

- If WSSV was established in the wild, spread to hatchery crustaceans could occur through use of WSSV vectors, harvested from areas where WSSV was established, such as polychaetes as feed for broodstock.
- If WSSV were to establish on a farm it could spread to neighbouring farms and wild populations through effluent water. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of WSSV be suspected and response measures initiated. However, WSSV is effectively transmitted through water, and farms which share a common water source with an infected population may be exposed to WSSV. WSSV can remain infective in the water column for some time.
- The likelihood of WSSV spread from farms to wild populations is greater than for other hazards with limited host ranges and few non-prawn vectors, for example, infectious myonecrosis virus. It is assumed that all decapod crustaceans exposed to WSSV would be susceptible to infection.
- The likelihood of WSSV spread from farms to wild populations or neighbouring farms via escaped prawns is reduced due to the systems in place on farms to prevent discharge of live animals, however WSSV could spread this way.
- The likelihood of spread of WSSV from hatchery crustaceans to wild crustaceans is reduced because of the closed systems, stronger biosecurity procedures and water treatment in place for these facilities. However, because the host range is so broad for WSSV, it is considered more likely than for other hazards.
- Spread of WSSV from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae as all prawn species cultured in Australia are susceptible to WSSV. WSSV is less likely to spread this way than hazards which do not show clinical signs or high mortality. In addition, the comprehensive protocols for transferring postlarvae should minimise the likelihood of spread of WSSV from a hatchery to a farm.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of WSSV in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**High**.
- Hatchery crustaceans—**Moderate**.
- Wild crustaceans—**High**.

Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of WSSV were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- Australia's farmed crustacean species, including prawns, yabbies and crayfish, are susceptible to WSSV. There is high morbidity and mortality associated with infection.
- Annual production losses due to WSSV have traditionally equated to approximately one tenth of global prawn production (Stentiford et al. 2012).

- In Australia, for example, the Logan River prawn farming industry production losses in 2016–17 were estimated to be approximately AU$23.5 million (excluding their response costs) and it was estimated that the cost of lost hatchery and breeding stocks were AU$5–6 million.
- WSSV has been detected in wild prawn populations but there are no reports of declines in catch rates or significant levels of associated mortalities (Diggles 2020; Hood et al. 2019; Muhammad et al. 2020; Orosco & Lluisma 2017; Saravanan et al. 2017). If WSSV were to establish in wild crustacean species that are used for commercial fisheries (such as prawns, crabs or rock lobsters), high mortalities in those populations would affect the catch rate, resulting in production losses for those industries.
- Based on the impacts seen in Australia and the rest of the world due to WSSV, WSSV establishment and spread in Australia would be expected to have a significant impact at the national level on life or health of susceptible animals.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- WSSV has been detected in the wild and has a wide host range (OIE 2021k). There are no reports of serious effects of WSSV infection in wild crustacean populations overseas. However, if WSSV were to establish and spread to the wild, there could be a significant impact on native species due to all decapod crustaceans found in Australia being susceptible.
- The direct impact of WSSV establishment and spread on the environment is expected to be minor at the state or territory level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- WSSV is listed as a disease notifiable to the WOAH and is included on Australia's *National list of reportable diseases of aquatic animals*. State and territory governments would be expected to report on the agent. There is an AQUAVETPLAN manual for WSSV and an Emergency Animal Disease response plan in the event of a WSD outbreak.
- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating WSSV from wild crustacean populations is unlikely to be launched. During the outbreak in the prawn farms on the Logan River, Queensland, eradication of WSSV was not attempted in the wild (only on the prawn farms) and to date has not resolved on its own.
- If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely.
- Eradication and control costs for a WSD outbreak are significant. When the WSD outbreak in the prawn farms on the Logan River in South-East Queensland occurred, the Commonwealth and Queensland governments spent more than AU$47 million in response, eradication and control programs. Due to the ongoing nature of the outbreak, these costs are increasing. In addition, to demonstrate that eradication is successful, there needs to be a national surveillance exercise over two years to confirm Australia's freedom from WSSV, at considerable cost.
- Eradication of WSSV causes, at least, minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Other industries such as seafood suppliers, commercial wild catch fisheries and the bait industry may be affected due to the host range of WSSV including polychaetes, prawns and all decapod crustaceans.

- Effects can also occur in all species potentially capable of transmitting the virus which may be indirectly affected by movement regulated areas. For example, in Australia, during the 2016–17 WSD outbreak in Queensland, the impact of the movement regulated area on the fisheries industry was estimated to be AU$20.5 million. As the movement regulated area remain in place these costs are ongoing
- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted, or decreased, feed companies would be impacted by reduced feed purchases.
- WSD affected prawns may show gross signs which could affect their marketability.
- WSSV establishment and spread would likely have a minor impact at the national level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- WSSV is a WOAH-listed disease. Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. WSSV establishment and spread may result in loss of some export markets due to importing country biosecurity requirements.
- If WSSV were to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product. However, export markets for prawns farmed or fished from the affected zones may be lost or restricted, and access to new markets could be impacted.
- WSSV is widespread throughout the world and it is assumed the effect on trade may be minor.
- The impacts of WSSV establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- WSSV has been detected in wild prawn populations and has a wide decapod host range (OIE 2021k). Many of these species are abundant and widely distributed in waters around Australia. WSSV may have an impact on the survival of these species which may affect biodiversity.
- The department lists five crustacean species as critically endangered, five as endangered and three as vulnerable. These are decapod crustaceans and are expected to be susceptible to WSSV infection; it is not known whether they would be susceptible to clinical disease.
- A conservative approach has been adopted in light of this uncertainty and when considering the susceptibility of native species, particularly those that are endangered or threatened.
- The impact of WSSV establishment and spread on the biodiversity of the environment is considered to be minor at the national level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Freshwater and marine crustaceans that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of WSSV which may impact on social amenity. This includes impacts on important species for indigenous cultural fishing, such as yabbies.

- In local areas where prawn farming is a major industry, a WSSV outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of WSSV establishment and spread are expected to be minor at the district or region level.

Table 20 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of WSSV. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 20 Overall impact of establishment and spread of WSSV for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Significant	F
	The environment (native animals/plants, and non-living environment)	State or territory	Minor	D
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	National	Minor	E
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	National	Minor	E
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	C

Conclusion
The overall impact of establishment and spread of WSSV was estimated to be **high**.

Determination of likely consequences of the outbreak scenario
The likely consequences of the outbreak scenario for WSSV in each exposure group were calculated by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**High**.
- Hatchery crustaceans—**High**.
- Wild crustaceans—**High**.

14.3.5 Determination of partial annual risk

The partial annual risk of WSSV entry, establishment and spread for each exposure group was calculated by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Low**.
- Hatchery crustaceans—**High**.
- Wild crustaceans—**High**.

14.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with WSSV in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption is **extreme**.

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

14.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for WSSV in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 14.4.1 to 14.4.6 present the factors considered and the conclusions reached.

14.4.1 Head and shell removal

When determining if head and shell removal would reduce the overall risk of WSSV to meet Australia's ALOP, the key points considered were:

- WSSV occurs throughout the whole prawn body.
- Head and shell removal is not expected to reduce the likelihood of entry of WSSV because sufficient WSSV would still be present in the tail to infect susceptible species. WSSV loads of about 1.9×10^{10} WSSV copies/g of tissue are present in the tail muscle (Durand et al. 2003), an amount that is considered sufficient to cause an infection in susceptible animals.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Head and shell removal is expected to further reduce the likelihood of this occurring because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed for aquatic animals held in research or public aquaria.
- Whole uncooked prawns are preferred over other forms (including uncooked and shelled) of prawns for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal, applied was determined to be **high**.

Therefore, as the overall restricted risk does not achieve Australia's ALOP, additional specific biosecurity measures are considered necessary for this hazard.

14.4.2 Head and shell removal plus deveining

When determining if head and shell removal plus deveining would reduce the overall risk of WSSV to meet Australia's ALOP, the key points considered were:

- Head and shell removal plus deveining is not expected to reduce the amount of WSSV present in the imported prawn and therefore the likelihood of entry of WSSV.
- The additional step of deveining is not expected to cause a reduction in exposure likelihoods for farmed or hatchery crustaceans compared to prawns which have only had the head and shell removed.
- The additional step of deveining is also not expected to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley compared to prawns which have only had the head and shell removed. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal plus deveining. Additionally, the very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels remains.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal plus deveining, applied was determined to be **high.**

Because the application of deveining (when applied in combination with head and shell removal) does not reduce the overall restricted risk compared to head and shell removal-only, deveining is not considered further as a specific biosecurity measure for WSSV.

14.4.3 Head and shell removal in combination with pre-export batch testing

When determining if head and shell removal combined with pre-export batch testing would reduce the overall risk of WSSV to meet Australia's ALOP, the key points considered were:

- There are sensitive qPCR methods available to detect WSSV in prawns (OIE 2021k).
- Post-processing batch testing of prawns for WSSV prior to export (after the application of head and shell removal) would reduce the likelihood of entry from high to low. Under this scenario it is assumed that the testing is not conducted under a department approved testing or sampling system.
- Pre-export testing does not change the appearance of the prawns, therefore it is not considered to reduce the likelihood of them being used for unintended purposes (such as bait or berley), more than head and shell removal does. As such, the exposure likelihood with pre-export and on-arrival testing applied is consistent with the exposure likelihood of head and shell removal.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal in combination with pre-export batch testing, applied was determined to be **moderate.**

Therefore, as the overall restricted risk does not achieve Australia's ALOP, additional specific biosecurity measures are considered necessary for this hazard.

14.4.4 Head and shell removal in combination with pre-export and on-arrival batch testing

When determining if head and shell removal combined with pre-export and on-arrival batch testing would reduce the overall risk of WSSV to meet Australia's ALOP, the key points considered were:

- There are sensitive qPCR methods available to detect WSSV in prawns (OIE 2021k).
- Batch testing of prawns for WSSV on-arrival in Australia (after the application of head and shell removal and pre-export testing) would reduce the likelihood of entry from low to extremely low. Under this scenario, testing and sampling is being conducted under departmental control and oversight.
- Pre-export and on-arrival testing does not change the appearance of the prawns, therefore it is not considered to reduce the likelihood of them being used for unintended purposes (such as bait or berley), more than head and shell removal does. As such, the exposure likelihood with pre-export and on-arrival testing applied is consistent with the exposure likelihood of head and shell removal.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal in combination with pre-export and on-arrival batch testing, applied was determined to be **very low**.

14.4.5 Cooking

When determining if cooking would reduce the overall risk of WSSV to meet Australia's ALOP, the key points considered were:

- WSSV-positive prawns boiled at 100°C for 1, 3, 5, 10 and 30 mins were not capable of transmitting WSSV to healthy prawns following *per os* exposure (Aranguren et al. 2020b). In these experiments, it took approximately 48 seconds for the core temperature of the prawns to reach 70°C (Aranguren et al. 2020b).
- WSSV-infected prawns cooked to reach a core temperature of 60°C, 70°C, 75°C, 85°C, and 95°C were not capable of transmitting WSSV to healthy prawns following *per os* exposure (Aquaculture Pathology Laboratory & Department of Agriculture 2022a).
- Given the data about the effect of cooking on WSSV infectivity, cooking to attain a core temperature of at least 65°C is therefore expected to significantly reduce the likelihood of entry of infectious WSSV in imported prawns.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **very low**.

14.4.6 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of WSSV to meet Australia's ALOP, the key points considered were:

- Value-added products are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). The likelihood of entry of WSSV is expected to be the same as for head and shell removal. This is because it is not expected that the processing will further reduce the amount of viable WSSV in the product more than head and shell removal does.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

Conclusion
Based on this information, the overall restricted risk of imported frozen uncooked prawns which have been processed into a value-added product, was determined to be **very low**.

15 Yellow head virus genotypes 1 and 8 risk review

15.1 Background

Yellow head virus (YHV) genotype 1 (YHV1) is the aetiological agent of yellow head disease (YHD). While all genotypes in the yellow head complex appear able to infect prawns, YHV genotype 8 (YHV8) has been shown to cause significant disease in prawns and is speculated to have a similar virulence to YHV1 (Liu et al. 2014). The genotypes in the yellow head complex are formally classified by the International Committee on Taxonomy of Viruses (ICTV) in the family *Roniviridae* (Cowley et al. 2011; ICTV 2018). Host species susceptible to YHV1 and YHV8 include various penaeid and caridean prawns (OIE 2021l; Zhu et al. 2016).

YHD was first reported in Thailand in the early 1990s in *Penaeus monodon* (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993). However, it is believed to have emerged in Taiwan in the late 1980s (Chen & Kou 1989; Kibenge & Godoy 2016). YHV1 is now present in many countries of Asia and Mexico. To the best of knowledge, YHV8 has been reported from China where it was first isolated from diseased farmed prawns collected during 2012 and 2013 (Liu et al. 2014; Thitamadee et al. 2016; Zhu et al. 2016) and the Republic of Korea (Kim et al. 2020).

Infection with YHV1 is listed as a disease notifiable to the World Organisation for Animal Health (WOAH, formerly OIE) (OIE 2021c) and is on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Infection with YHV8 is not notifiable to the WOAH (OIE 2021c), is not on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020) and is not in the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2020c). YHV genotypes 2, 6 and 7 are present in Australia (Cowley et al. 2000; FRDC 2018; Mohr et al. 2015). Australia has a long history of passive surveillance and a strong system in place to detect incursions, YHV1 and YHV8 are considered exotic to Australia.

The only members of the yellow head complex which comply with the criteria described in the WOAH *Aquatic animal health code* (WOAH Code) Article 2.1.2 *Hazard Identification* (WOAH 2022c) and have been retained as hazards, are YHV1 and YHV8. This chapter will use information about all YHV genotypes where data are lacking for YHV1 and YHV8. Where it is unclear which genotype is being referred to in the literature the name used in the cited literature will be used. Note, if required, biosecurity measures will only be applied to YHV1 and YHV8.

15.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of YHV1 and/or YHV8 is warranted.

15.2.1 Agent properties

Yellow head complex virions are enveloped, rod-shaped particles 40–60nm × 150–200nm in dimensions (Cowley et al. 2011). Envelopes are studded with prominent peplomers projecting approximately 11nm from the surface. YHV is formally classified by the ICTV as a member of the genus *Okavirus*, in the family *Roniviridae* (Cowley et al. 2011). Gill associated virus (GAV), also known

as YHV genotype 2 (YHV2) is the type species for the genus (Cowley et al. 2011). Yellow head virus (species *Yellow head virus*) is assigned to genotype 1 and yellow head virus 8 (species *Okavirus 1*) is assigned to genotype 8. Viruses assigned to genotypes 3-7 have not yet been classified taxonomically as complete genome sequences are not yet available (Walker et al. 2020).

YHV1 has been subdivided into YHV Type-1a (YHV1a) and YHV Type-1b (YHV1b). YHV1b is characterized by a 162 bp deletion in the ORF3 region encoding the structural gene for gp116 when compared to YHV1a (Sittidilokratna et al. 2009a). However, despite this deletion there has not been any differences reported in the histopathology between infections and electron microscopy has revealed the virions are morphologically indistinguishable (Senapin et al. 2010). There is evidence of genetic recombination between genotypes (Wijegoonawardane et al. 2009).

YHV-infected tissues or extracts stored at –70°C (Lu et al. 1995) and –80°C (Direkbusarakom et al. 1998) remain infective. Infectious YHV has also been detected in frozen prawns sourced from retail outlets in the United States of America (Nunan, Poulos & Lightner 1998). YHV has been reported to survive in 25°C to 28°C seawater for at least 4 days (Cowley et al. 2011; Flegel et al. 1995). Freeze-thaw cycles (Wongteerasupaya et al. 1995a) and digestion in bird gut (Vanpatten, Nunan & Lightner 2004) may damage virions. Virions are sensitive to calcium hypochlorite and sodium dodecyl-sulfate but sensitivity to other treatments is not known (Cowley et al. 2011).

YHV1 is reported to be inactivated by heating at 60°C for 15–30 mins (Cowley et al. 2011; Flegel et al. 1995). The department commissioned the University of Arizona in the United States of America to conduct independent research to determine the minimum core temperature required to inactivate white spot syndrome virus and YHV1. YHV1-infected prawns were cooked to reach specific core temperatures ranging from 60°C to 95°C. They were then fed as minced cooked tissue to specific pathogen free *P. vannamei*. YHV1-infected prawns cooked to a core temperature of 60°C, 70°C, 75°C, 85°C, and 95°C were not capable of causing YHV1-infection in naive prawns (Aquaculture Pathology Laboratory & Department of Agriculture 2022a).

15.2.2 Epidemiology

Host range

Species which fulfil the criteria for listing as a species susceptible to infection (N=natural; E=experimental exposure) with YHV1 in accordance with chapter 1.5 of the WOAH Code (WOAH 2022c) include:

- *Metapenaeus affinis* [E] (prawn) (Longyant et al. 2006)
- *Palaemonetes pugio* [E] (prawn) (Ma, Overstreet & Jovonovich 2009)
- *Penaeus monodon* [N, E] (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993)
- *Penaeus stylirostris* [N, E] (Castro-Longoria et al. 2008; de la Rosa-Velez et al. 2006; Lightner 1996b; Lu et al. 1994)
- *Penaeus vannamei* [N, E] (de la Rosa-Velez et al. 2006; Lightner et al. 1998; Senapin et al. 2010; Sittidilokratna et al. 2009a).

Other host species shown to be susceptible to infection with YHV1 include (N = natural; E=experimental exposure):

- *Macrobrachium sintangense* [E] (Longyant et al. 2005)
- *Metapenaeus bennettae* [E] (prawn) (OIE 2010)
- *Metapenaeus brevicornis* [E] (Longyant et al. 2006)
- *Metapenaeus ensis* [E] (Chantanachookin et al. 1993; Flegel et al. 1995)
- *Palaemon serrifer* [E] (Longyant et al. 2005)
- *Palaemon styliferus* [E] (Flegel et al. 1995; Longyant et al. 2005)
- *Penaeus aztecus* [E] (Lightner 1996b; Lightner et al. 1998)
- *Penaeus duorarum* [E] (Lightner 1996b; Lightner et al. 1998)
- *Penaeus japonicus* [N] (Wang et al. 1996)
- *Penaeus merguiensis* [E] (Boonyaratpalin et al. 1993; Flegel et al. 1995)
- *Penaeus setiferus* [E] (Lightner 1996b; Lightner et al. 1998).

YHV1-positive RT-PCR results have also been reported in a few species (N=natural; E=experimental exposure), however no active infection has been demonstrated, including:

- *Acetes* species [N] (paste shrimp) (Chantanachookin et al. 1993; Flegel, Fegan & Sriurairatana 1995; Flegel et al. 1995)
- *Callinectes sapidus* [E] (blue crab) (Overstreet, Jovonovich & Ma 2009)
- *Chelonibia patula* [E] (acorn barnacle) (Overstreet, Jovonovich & Ma 2009)
- *Cherax quadricarinatus* [E] (Soowannayan et al. 2015)
- *Ergasilus manicatus* [E] (cyclopoid copepod) (Overstreet, Jovonovich & Ma 2009)
- *Fundulus grandis* [E] (Gulf killifish) (Overstreet, Jovonovich & Ma 2009)
- *Octolasmis muelleri* [E] (gooseneck barnacle) (Overstreet, Jovonovich & Ma 2009)
- *Portunus pelagicus* [E] (crab) (Boonsaeng et al. 2000)
- *Scylla serrata* [E] (crab) (Boonsaeng et al. 2000)
- *Sesarma* species [E] (crab) (Boonsaeng et al. 2000)
- *Uca spinata* [E] (crab) (Boonsaeng et al. 2000).

Species considered susceptible to infection (N= natural exposure) with YHV8 include:

- *Macrobrachium rosenbergii* [N] (Zhu et al. 2016)
- *Penaeus chinensis* [N] (Zhu et al. 2016)
- *Penaeus japonicus* [N] (Zhu et al. 2016)
- *Penaeus vannamei* [N] (Zhu et al. 2016).

YHV1 affects late postlarval stages, juvenile and adult prawns (OIE 2021l). *P. monodon* are susceptible to YHV1 beyond postlarvae 15 (OIE 2021l). In one study, YHV was transmitted to juvenile prawns by feeding infected tissues, but postlarvae were found to be resistant to infection (Lightner et al. 1998). During severe outbreaks, high prevalence of YHD is most common in farmed *P. monodon* 50–70 days after stocking, when prawns are in the juvenile to sub-adult stage (5–15g) (Lightner 1996a; Lotz 1997).

Geographical distribution

YHV1 has been reported in Taiwan, Indonesia, Malaysia, the Philippines, Sri Lanka, Thailand (Walker et al. 2001), Brunei Darussalam (NACA, OIE-RRAP & FAO 2021) and Mexico (de la Rosa-Velez et al. 2006; Sánchez-Barajas, Liñán-Cabello & Mena-Herrera 2009).

YHV8 has been detected in China (Liu et al. 2014) and was later reported in cultured prawns in the Republic of Korea (Kim et al. 2020).

Prevalence

The overall prevalence of yellow head complex viruses can be 50–100% in healthy *P. monodon* in farmed and wild populations in Australia, Asia, and East Africa and in farmed *P. vannamei* in Mexico (Cowley et al. 2004; OIE 2021l; Sánchez-Barajas, Liñán-Cabello & Mena-Herrera 2009; Walker et al. 2001; Wijegoonawardane et al. 2008). The prevalence of individual genotypes varies according to the geographic origin of the prawn (OIE 2021l). The use of detection methods less sensitive than nested PCR will likely result in an underestimation of the prevalence amongst populations of prawns being investigated (OIE 2021l).

YHV1 prevalence may be less than 1% in healthy wild or farmed *P. monodon* but its prevalence would approach 100% in farmed prawns undergoing YHD outbreaks (OIE, 2006 cited in (Stentiford, Bonami & Alday-Sanz 2009)). A simple pond prevalence analysis of various prawn pathologies in 196 randomly selected ponds in Thailand during 2013–2014 did not find YHV1 in sampled prawns using RT-PCR (Sanguanrut et al. 2018). Similarly, in farmed populations the apparent prevalence of YHV in *P. vannamei* farmed in greenhouse ponds in China was found to be 0% (Shen et al. 2017).

There are few reports of the prevalence of YHV1 in wild prawn populations. An epidemiological study of 230 wild *P. monodon* collected in Thailand found 46% (105/230) were positive for YHV1 (Hamano et al. 2017). YHV1 was detected at 4% prevalence (2/52) in clinically normal wild *P. stylirostris* collected for surveillance purposes in the Gulf of California in 2003 (Castro-Longoria et al. 2008).

Two surveys of farmed prawns in provinces of China have reported a prevalence of YHV8 infection ranging from 9.5% (14/147) (Yang et al. 2016) to 11% (33/299) (Zhu et al. 2016). In both studies, *P. chinensis* showed the highest YHV8 infection rates (52.3% and 75%) (Zhu et al. 2016). In the Republic of Korea it was reported that YHV8 was detected in 9% (21/234) of *P. vannamei* and 100% (17/17) of *P. chinensis* obtained from 7 farms (Kim et al. 2020).

No reports were found on the prevalence of YHV8 in wild prawn populations.

Mortalities

Infection with YHV1 is associated with rapid accumulation of mortalities (up to 100% within 3–5 days of the first appearance of clinical signs) during disease outbreaks (OIE 2021l). YHD was reported to have caused extensive mortalities of *P. monodon* when it first emerged in Thailand in 1990 and 1991 (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993). A study of 20 *P. vannamei* farms in Thailand showing gross signs of YHD in 2007-2008 reported a cumulative mortality of 60–70% (Senapin et al. 2010). Although YHD remains an enzootic disease in Asia, mortalities due to YHD are now rarely reported (Lightner et al. 2012b).

Transmission

YHV1 infection can be transmitted by injection, ingestion of infected tissue, immersion of healthy prawns in sea water containing filtered tissue extracts, and by cohabitation of healthy prawns with infected prawns (Flegel et al. 1995; Lightner 1996b). *P. monodon* exposed to YHV1 by cohabitation with experimentally infected crabs or infected red claw crayfish also developed YHD despite physical separation of the animals (Boonsaeng et al. 2000; Soowannayan et al. 2015). Hamano et al. (2015) evaluated the role of direct and indirect contact with YHV in *P. monodon* and reported that cannibalism of moribund prawns represented a far greater potential to transmit YHD than water exposure alone. The rapid accumulation of mortalities during disease outbreaks suggests that horizontal transmission occurs very effectively (OIE 2021l).

Transmission from broodstock to progeny has not been demonstrated for YHV1; however, it is suggested to occur as other viruses in the yellowhead complex such as GAV are transmitted from broodstock to progeny (Cowley et al. 2002). Surviving experimentally infected prawns have been shown to be hosts of YHV without showing clinical signs (Longyant et al. 2005).

C. quadricarinatus exposed to YHV1 through direct inoculation, feeding on infected prawns or cohabitation with infected prawns were shown to be positive for YHV1 by RT-PCR and were able to transmit the virus as demonstrated through transmission bioassays. They did not however show any gross or histopathological signs of YHD (Soowannayan et al. 2015). These results indicate that *C. quadricarinatus* may be a vector for YHV. Paste prawns (*Acetes* species) may be vectors of YHV as extracts of paste prawns collected from YHV-infected ponds could cause YHD when injected into *P. monodon* (Flegel, Fegan & Sriurairatana 1995; Flegel et al. 1995). Mechanical vectors such as infected transport water, intake water, nets and other equipment may also be sources of YHV (Stentiford, Bonami & Alday-Sanz 2009).

An unpublished on-farm study in Thailand cited in Senapin et al. (2010) concluded that YHV is spread by an airborne vector. A follow-up unpublished study cited in Thitamadee et al. (2016) tested a wide variety of potential vectors from the farm environment including crustaceans, molluscs, insects, insect larvae and other organisms in the aquatic environment. However, no positive results were obtained by RT-PCR for these species. Another study investigating parasitic crustaceans as vectors of prawn viruses, including YHV, found that the level of YHV in crustacean parasites on fish and crabs decreased over the 2 weeks of the study following exposure to YHV through the water column (Overstreet, Jovonovich & Ma 2009).

Mechanism of spread

The spread of YHV has mostly been attributed to the uncontrolled introduction of live prawn stocks and subsequent unrestricted movement of live broodstock and postlarvae (Briggs et al. 2004). It has been speculated that the international trade of frozen raw prawns may facilitate the introduction of prawn viruses into new areas through the inappropriate disposal of processing and retail wastes, the use of imported prawns for bait and inadequately processed prawn feeds resulting in possible pathways of YHV exposure for farmed and wild crustaceans (Durand, Tang & Lightner 2000; Humphrey 1995; Lightner 1995).

Infectious dose

The minimum infectious dose of YHV1 required to cause YHD in susceptible species by experimental challenge or natural infection is not known. However, *per os* bioassays showed that YHV1 has been

successfully transmitted to juvenile *P. vannamei* fed on 0.6g pieces (days 0, 2, 4 and 6) of YHV-infected prawn carcasses (Lightner et al. 1998). In experimental challenge trials, YHV1 viral loads of 3.8×10^{10}–1.5×10^{11} RNA copies/ml resulted in 100% cumulative mortality of *P. vannamei* 6 days post-injection (Sittidilokratna et al. 2009a). In experiments by Sritunyalucksana et al. (2010), 10g *P. monodon* injected with 2.7×10^6 YHV viral copies/g of prawn resulted in 100% mortality of prawns within 48 hours. *P. monodon* injected with approximately 10^6 viral copies died within 3–4 days post-injection (Hamano et al. 2015).

15.2.3 Pathogenesis

Infections with YHV may be chronic or acute. In acute infections associated with disease and mortalities, YHV invades most tissues of ectodermal and mesodermal origin (Cowley et al. 2011). In chronic and subclinical infections, YHV pathology is mainly limited to the lymphoid organ (Anantasomboon et al. 2008b; Boonyaratpalin et al. 1993; Cowley et al. 2011). YHV8 has been associated with disease and is suspected to have a similar virulence to YHV1 (Thitamadee et al. 2016; Zhu et al. 2016)

Duangsuwan et al. (2011) reported a putative pathway for YHV infection in the lymphoid organ following transmission electron microscopy studies in experimentally infected *P. monodon*. It was proposed that YHV viral particles enter stromal cells and haemocytes in the lymphoid tubule walls by endocytosis and become uncoated. YHV then pass into the cytoplasm, where the viral genomes are replicated, the nucleocapsid proteins are synthesized and the viral envelopes are formed. The completely enveloped viral particles are then packaged in secretory vesicles and released by exocytosis at the cell membrane (Duangsuwan et al. 2011).

YHV infection is often reported as a co-infection with white spot syndrome virus (WSSV) and other viruses (Durand, Tang & Lightner 2000; Madhavi et al. 2002; Mohan et al. 1998; Wang & Chang 2000). A viral interference effect between Taura syndrome virus (TSV) and YHV was suggested following experimental infections showing that specific pathogen free *P. vannamei*, which were pre-exposed to TSV and then challenged with YHV, acquired partial protection from YHD (Aranguren, Tang & Lightner 2012).

Tissue tropism

YHV affects tissues of ectodermal and mesodermal origin, particularly the lymphoid organ, haemocytes, hematopoietic tissue, connective tissues, cuticular epithelium, gills, epicardium, antennal gland, gonads and nerve tissues, including neural ganglia, nerve fibres and glial cells (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993; Lightner 1996b; Tang & Lightner 1999; Wongteerasupaya et al. 1995b).

Specific interaction between YHV and granule-containing haemocytes has been reported, however it is unknown whether these cells are one of the primary targets of YHV or are the first line of viral defence (Havanapan et al. 2016).

Tissue titre

According to Sritunyalucksana et al. (2010), 5×10^5 viral copies/g of prawn are reported to represent a pre-patent viral load and 2500 viral copies/g of prawn represent a viral load in grossly normal hosts. *P. monodon* intramuscularly injected with YHV had 7.75×10^6 viral RNA copy numbers in the haemolymph 48 hours post-infection (Soowannayan et al. 2013).

15.2.4 Diagnosis

Clinical signs

YHD is characterised by cessation of feeding, swimming slowly near the surface at the edges of ponds, quickly followed by high mortality of up to 100% over a period of 3–5 days (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993). Affected prawns are pale bodied (bleached appearance) with reddening of the appendages, and have a yellow cephalothorax due to a yellow hepatopancreas visible through the translucent carapace (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993). Yellowing of the cephalothorax does not always occur in affected animals and is not typical for all species (Chantanachookin et al. 1993; Lu et al. 1994; Tang & Lightner 1999). The clinical signs are most commonly observed and the mortality rate is the highest during the juvenile to sub-adult stage (Lightner 1996b). Many infections are also subclinical (Castro-Longoria et al. 2008; OIE 2021l).

Pathology

Systemic infection causes extensive necrosis in ectodermal and mesodermal tissues with intense basophilic, cytoplasmic and spherical inclusions (Flegel, Boonyaratpalin & Withyachumnarnkul 1997). Haemocytes from smears display pyknotic and karyorrhectic nuclei (Lu et al. 1994; Nash, Arkarjamon & Withyachumnarnkul 1992).

Testing

Chapter 2.2.9 of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH 2022k) provides details of the methods currently available for targeted surveillance and diagnosis of YHV1.

Nested RT-PCR followed by confirmatory sequencing of the amplified PCR product is the recommended method for targeted surveillance to declare freedom from YHV1 (WOAH 2022k).

RT-PCR (Mohr et al. 2015; Wongteerasupaya et al. 1997), nested RT-PCR (Cowley et al. 2004; Mohr et al. 2015; Wijegoonawardane et al. 2008) and qRT-PCR (Dhar, Roux & Klimpel 2002; Wijegoonawardane, Cowley & Walker 2010) can be used to detect YHV1. *In situ* hybridisation (Tang & Lightner 1999), Western blot (Flegel 1998; Soowannayan et al. 2003) and real-time reverse-transcription loop-mediated isothermal amplification (rRT-LAMP) (Mekata et al. 2006; Yang et al. 2016) are also available for detecting YHV1.

YHV8 can be detected by RT-PCR and nested RT-PCR (Mohr et al. 2015; WOAH 2022k). A one-step, rRT-LAMP assay has been described for detection of YHV8 (Yang et al. 2016).

15.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments (WOAH 2022k).

15.2.6 Control

General control measures to prevent infections include restricting the movement of live broodstock and postlarvae, use of specific pathogen free broodstock, screening of broodstock and postlarvae as YHV1-negative before pond stocking, enforcement of codes of conduct and management practices, improving husbandry technology in intensive aquaculture and active surveillance (Briggs et al. 2004). Strategies for limiting spread of the virus include strict hygiene procedures, disinfection of ponds and water inlet channels, use of only dry commercial feeds and fine screening of inlet water to eliminate carrier prawns (Flegel et al. 1995). RNA interference (RNAi) has been used experimentally as a

method to control YHV1 infection. For example, injection of prawns with double stranded RNA homologous to YHV1 has been found to inhibit viral replication and prevent mortalities following challenge trials (Assavalapsakul, Chinnirunvong & Panyim 2009; Tirasophon et al. 2007).

15.2.7 Impact of the disease

YHD was widely reported as the first major virulent disease threat to *P. monodon* aquaculture (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993). Total production loss attributed to YHV during the initial outbreak in Thailand in the early 1990s was estimated at US$30–200 million or approximately 3% of total production volume (Flegel et al. 1995). Two outbreaks of YHD (in combination with white spot disease) in India in 1994 and 1995 resulted in production losses of 10,000–12,000 tonnes (Mohan and Basavarajappa 2001 cited in (Shinn et al. 2018b)). Infection with YHV1 in Asia is estimated to have resulted in US$0.5 billion production losses (Lightner et al. 2012b).

There is little recent quantitative data on the economic consequences of YHD outbreaks. Senapin et al. (2010) reported the economic losses estimated by the Thai Animal Aquaculture Association for YHD outbreaks in farmed *P. vannamei* in two provinces in Thailand from late 2007 to early 2008, to be approximately US$3 million.

It has been reported that production volumes following an outbreak of YHD return to pre outbreak levels over a relatively short period (Flegel 1997a, b). Mortalities due to YHD in Thailand were initially serious and widespread. However, high level mortality of *P. monodon* attributed to YHD declined within 1.5 years (Flegel 1997b).

Although YHV1 has been detected in wild prawns (Hamano et al. 2017), no reports were found about the impact of YHV1 on wild crustacean populations. Similarly, no reports were found about the impact of YHV8 on wild prawn populations.

15.2.8 Current biosecurity measures

The Prawn IRA 2009 determined the unrestricted risk associated with YHV1 to be high and therefore biosecurity measures were necessary (Biosecurity Australia 2009).

Current biosecurity measures which manage risks for YHV1 are:

- demonstration of source population freedom
- cooking
- highly processed prawn products (dumpling and dim sum type-products)
- breaded, battered or crumbed prawns
- head and shell removal (last segment and tail fan excluded) in combination with pre-export and on-arrival testing.

YHV8 was not assessed in the Prawn IRA 2009 and there are no current biosecurity measures specific for YHV8.

15.2.9 Conclusion

YHV1 is present in exporting countries, is not present in Australia and is capable of causing adverse effects in Australia. In Australia, infection with YHV1 is a nationally notifiable disease. Based on the preceding information, risk assessment for YHV1 is warranted.

The department considers there is insufficient information regarding YHV8 to conduct a risk assessment and will continue to monitor the situation with respect to YHV8. The department routinely analyses ongoing media and scientific literature about biosecurity issues for all animal species to monitor biosecurity risks. The scientific information is regularly assessed by technical experts and if new information about a biosecurity risk is identified, the department reviews the risk further and acts when necessary. The department could include YHV8 in ongoing monitoring (see section 17.4.2 Ongoing monitoring of batches of imported uncooked prawns) of imported prawns should new information become available about YHV8 which suggests ongoing monitoring would provide relevant information. Should new information become available about YHV8, the department will consider the information and if appropriate, a risk assessment specific for YHV8 will be conducted.

15.3 Risk assessment

Based on chapter 4 and the technical information about YHV1 presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of YHV1 meets Australia's ALOP are shown in Appendix F.

15.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for YHV1 were that:.

- This draft risk review is generic and therefore the entry assessment assumes that YHV1 is present in all source countries.
- YHV1 infects various penaeid and caridean prawn species of marketable size that are exported to Australia.
- Prevalence of YHV1 in farmed and wild prawns is variable, but can be up to 100%.
- YHV1 would be present in the whole body of infected prawns.
- The viral load of YHV1 in infected imported prawns is likely to be sufficient to cause infection in susceptible species.
- Post-harvest inspection may detect grossly abnormal prawns that are infected with YHV1 and remove them before export. Prawns with mild gross signs or which do not show clinical signs would be unlikely to be detected.
- YHV1 in imported prawns is expected to survive freezing, storage and transport and remain infectious at the time of import.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the annual likelihood of entry of YHV1 in imported prawns was estimated to be **high.**

15.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for YHV1 were that:

- YHV1 would be present in the whole body of infected prawns or in associated wastes that may enter the environment of the exposure groups.

- YHV1 would be expected to be present in sufficient loads in imported prawns (or associated wastes) to cause infection in susceptible species if exposed.
- YHV1 in imported prawns (or associated wastes) is likely to persist and remain infectious at the point of exposure.
- Important aquaculture and wild-caught species in Australia that are susceptible to infection with YHV1 include *P. monodon*, *P. merguiensis* and *P. japonicus*. Other YHV1 susceptible species and potential vectors are widespread in Australian waters, including *C. quadricarinatus*. The impact of YHV1 on threatened native Australian species such as the critically endangered *Cherax tenuimanus* is unknown.
- Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustaceans would make contact with, and likely be consumed by susceptible species in these exposure groups.
- Farmed crustaceans are considered unlikely to be intentionally fed imported prawns.
- Unintentional introduction into a farm may occur by imported prawns being used as bait in and around farm inlet and outlet channels. This would be a direct pathway to farmed crustaceans if a farm has not implemented standards of entry-level biosecurity for intake water that would exclude YHV1 or prawns used as bait or berley. Although this is considered a remote likelihood for direct exposure as most imported prawns being used as bait would be consumed by non-susceptible species before entering ponds.
- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns (or associated wastes) because hatchery biosecurity measures should prevent the use of imported prawns as feed and physical containment should prevent exposure to imported prawns used as bait and berley. However, it is assumed that a very small, yet significant volume of whole uncooked prawns would be used as feed for crustaceans in public aquaria and research facilities. Species susceptible to YHV1 are likely to be present in research facilities and public aquaria.
- Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Despite this, wild crustaceans are the most likely group to be directly exposed to imported prawns because of the repeated use of prawns as bait or berley by recreational fishers and due to the wide host range of YHV1 susceptible species present in Australian waters.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 7, the partial likelihood of exposure of each exposure group to YHV1 in imported prawns was estimated to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**High**.

15.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to YHV1 in imported prawns was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Farmed crustaceans—**Very low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**High**.

15.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)

The key points considered relevant when determining the partial likelihood of establishment and spread for YHV1 were that:

- YHV1 can be transmitted by ingestion of infected tissues, cohabitation and via water and can remain infectious in water for an extended period. YHV1 may be transmitted from broodstock to progeny.
- It is expected that susceptible species feeding on YHV1-infected prawns would receive an infectious dose.
- Prawns that survive YHV1 infection can remain infectious and become sources of the virus.
- Potential vectors of YHV1 are present in Australia and include crabs, barnacles and copepods.
- The main aquaculture and wild-caught species in Australia are susceptible to YHV1, including *P. monodon, P. merguiensis* and *P. japonicus* and are widespread in Australian waters. *C. quadricarinatus* is present in the wild and may act as a vector.
- The likelihood of YHV1 establishment, following a given quantity of YHV1 entering the environment of an exposure group, is the greatest for farmed crustaceans. This is due to several factors associated with intensive husbandry. For example, the higher density of susceptible animals, the environmental conditions associated with intensive husbandry practices and the absence of predators.
- If establishment of YHV1 were to occur in the wild, spread to other populations would be less likely than for farmed crustaceans because infected wild animals (particularly those clinically affected) are likely to be eaten by non-susceptible animals. The densities of susceptible and infected animals are also much lower which reduces the opportunities for transmission. However, the likelihood of YHV1 in a wild population spreading to its natural geographic limits is greater than for other hazards with limited host ranges and few non-prawn hosts, for example, infectious myonecrosis virus. The ability of prawns to remain infectious and become sources of YHV1 after surviving an infection also aids its spread.
- If YHV1 were to establish in the wild, especially in waters around prawn farms, it may easily spread to farms due to being transmissible through water. In the absence of effective biosecurity measures, wild infected prawns may be transferred into the farms through the inlet water channels. There are crustacean species from which YHV1 has been detected by PCR, for example *S. serrata*, and which can enter farms through movement across short distances of land and could potentially carry YHV1 with them.
- If YHV1 were to establish on a farm it could spread to neighbouring farms and wild populations through effluent water. This spread may be moderated by dilution effects and implementation of biosecurity measures should an incursion of YHV1 be suspected and response measures initiated. However, YHV1 is effectively transmitted through water, and farms which share a common water source with an infected population may be exposed to YHV1.
- The likelihood of YHV1 spread from farms to wild populations or neighbouring farms via escaped prawns is reduced due to the systems in place on farms to prevent discharge of live animals, however YHV1 could spread this way.

- If YHV1 were to establish in hatchery crustaceans, spread to wild crustaceans would be unlikely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.
- Spread of YHV1 from hatchery crustaceans to farmed crustaceans may occur through the movement of postlarvae, given *P. monodon* and *P. merguiensis* are both susceptible to YHV1. YHV1 is likely to be effectively transferred between hatchery and farm because postlarvae may not show clinical signs of infection until after transfer.

Conclusion

Based on these considerations and using the descriptors in Table 7, the partial likelihood of establishment and spread of YHV1 in each exposure group for the outbreak scenario (refer section 4.5.1 Identification of the outbreak scenario) was estimated to be:

- Farmed crustaceans—**High**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Low**.

Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of YHV1 were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- Australia's main farmed prawn species are susceptible to YHV1. There is high morbidity and mortality associated with infection.
- YHV1 would not be expected to impact wild fisheries in Australia. There are limited reports of YHV1 in wild prawns and no reports of declines in catch rates or associated mortalities.
- Based on the impacts in Asia from YHV1 infection, YHV1 establishment and spread in Australia would be expected to cause significant impacts at the national level on life or health of susceptible animals.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- Based on the absence of serious effects of wild prawn populations overseas, and the absence of any known impact of endemic YHV genotypes on wild prawn populations, the environmental effects of YHV1 establishment and spread are expected to be limited.
- Non-prawn crustaceans such as crabs, barnacles, copepods and paste shrimp (*Acetes* spp.) found in Australia may act as YHV1 vectors as they show no signs of infection.
- The direct impact of YHV1 establishment and spread on the environment is expected to be minor at the local level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with YHV1 is listed as a notifiable disease by the WOAH and is included on Australia's *National list of reportable diseases of aquatic animals*. State and territory governments would be expected to report on the agent.

- Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating YHV1 from wild crustacean populations is unlikely to be launched.
- If infected animals were considered likely to be confined to an aquaculture facility (farmed or hatchery), then an attempt at eradication is more likely.
- If a movement regulated area were put in place for an outbreak of YHV1, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- To demonstrate that eradication is successful, there would need to be a national surveillance exercise over at least two years to confirm freedom, at considerable cost.
- Eradication of YHV1 is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Other industries such as seafood suppliers, commercial wild catch fisheries, other crustacean industries and the bait industry may be affected due to the host range of YHV1.
- Effects can also occur in all potential YHV1 susceptible species which may be indirectly affected by movement regulated areas.
- Industries supplying inputs into the affected prawn regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- YHV1 infected prawns may show gross signs which could affect their marketability.
- YHV1 establishment and spread would likely have a minor impact at the national level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Infection with YHV1 is a WOAH-listed disease. Several countries have strong import requirements or have closed their borders to the importation of live, fresh and frozen prawns to avoid the introduction of prawn diseases. YHV1 establishment and spread may result in loss of some crustacean export markets due to importing country biosecurity requirements.
- If YHV1 were to become established, Australia could use zoning to maintain or gain access to international markets for live crustaceans including prawns and, if required, non-viable product.
- The impacts of YHV1 establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- YHV1 has a wide host range including *C. quadricarinatus,* although infection of this species does not cause clinical signs.
- It is unknown if *Cherax tenuimanus* is susceptible to infection with YHV1. However, *C. tenuimanus* is listed as critically endangered, and if YHV1 were to cause disease in *C. tenuimanus* it could have a significant impact on the survival of this already endangered species.

- In light of the uncertainty surrounding the susceptibility of *C. tenuimanus* to infection with YHV1, a conservative approach has been adopted when considering the susceptibility of native species, particularly those that are endangered or threatened, and it is assumed they are susceptible.
- The impact of YHV1 establishment and spread on the biodiversity of the environment is considered to be minor at the national level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Freshwater and marine crustaceans that are recreationally fished in Australia could be affected by movement regulated areas put in place due to an outbreak of YHV1 which may impact on social amenity.
- The social impacts of YHV1 establishment and spread are expected to be minor at the district or region level.

Table 21 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of YHV1. The individual impact scores were combined using the rules in Table 10 to estimate the overall impact (refer section 4.5.6 Determining impacts for detailed methodology).

Table 21 Overall impact of establishment and spread of YHV1 for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Significant	F
	The environment (native animals/plants, and non-living environment)	Local	Minor	B
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	National	Minor	E
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	National	Minor	E
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	C

Conclusion

The overall impact of establishment and spread of YHV1 was estimated to be **high**.

Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for YHV1 in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 5) and found to be:

- Farmed crustaceans—**High**.
- Hatchery crustaceans—**Moderate**.
- Wild crustaceans—**Moderate**.

15.3.5 Determination of partial annual risk

The partial annual risk of YHV1 entry, establishment and spread for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 6 and found to be:

- Farmed crustaceans—**Low**.
- Hatchery crustaceans—**Low**.
- Wild crustaceans—**Moderate**.

15.3.6 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 11.

The overall annual risk associated with YHV1 in non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption was found to be **moderate.**

Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard.

15.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for YHV1 in imported prawns to a level that meets Australia's ALOP are shown in Appendix F.

Sections 14.4.1 to 14.4.6 present the factors considered and the conclusions reached.

15.4.1 Head and shell removal

When determining if head and shell removal would reduce the overall risk of YHV1 to meet Australia's ALOP, the key points considered were:

- Head and shell removal is not expected to reduce the likelihood of entry of YHV1. This is because YHV1 infects tissues throughout the whole prawn. Whilst head and shell removal would reduce the viral load in the prawn, sufficient YHV1 to cause infection in a susceptible species following exposure is expected to remain.
- Head and shell removal is expected to reduce the likelihood of deliberate exposure of farmed and hatchery crustaceans because it removes the nutritional benefit associated with head-on prawns being used for maturation purposes. There may be some minor use of prawns which have had the head and shell removed as feed in research or public aquaria.
- Whole uncooked prawns are preferred over other forms (including uncooked and shelled) of prawns for use as bait by recreational fishers in Australia (Moore et al. 2022). Taking this into account, head and shell removal is considered to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is a reduction in the likelihood of exposure of wild susceptible species to imported prawns due to head and shell removal. There remains a very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal, applied was determined to be **moderate.**

Therefore, as the overall restricted risk does not achieve Australia's ALOP, additional specific biosecurity measures are considered necessary for this hazard.

15.4.2 Head and shell removal plus deveining

When determining if head and shell removal plus deveining would reduce the overall risk of YHV1 to meet Australia's ALOP, the key points considered were:

- Head and shell removal plus deveining is not expected to reduce the amount of YHV1 present in the imported prawn and therefore the likelihood of entry of YHV1.
- The additional step of deveining is not expected to cause a reduction in exposure likelihoods for farmed or hatchery crustaceans compared to prawns which have only had the head and shell removed.
- The additional step of deveining is also not expected to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley compared to prawns which have only had the head and shell removed. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal plus deveining. Additionally, the very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels remains.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal plus deveining, applied was determined to be **moderate.**

Because the application of deveining (when applied in combination with head and shell removal) does not reduce the overall restricted risk compared to head and shell removal-only, deveining is not considered further as a specific biosecurity measure for YHV1.

15.4.3 Head and shell removal in combination with pre-export batch testing

When determining if head and shell removal combined with pre-export batch testing would reduce the overall risk of YHV1 to meet Australia's ALOP, the key points considered were:

- Sensitive qRT-PCR methods are available to detect YHV1 in prawns (Wijegoonawardane, Cowley & Walker 2010).
- Post-processing batch testing of prawns for YHV1 prior to export (after the application of head and shell removal) would reduce the likelihood of entry from high to low. Under this scenario it is assumed that the testing is not conducted under a department approved testing or sampling system.
- Pre-export testing does not change the appearance of the prawns, therefore it is not considered to reduce the likelihood of them being used for unintended purposes (such as bait or berley), more than head and shell removal does. As such, the exposure likelihood with pre-export and on-arrival testing applied is consistent with the exposure likelihood of head and shell removal.

Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, head and shell removal combined with pre-export batch testing, applied was determined to be **low**.

Therefore, as the overall restricted risk does not achieve Australia's ALOP, additional specific biosecurity measures are considered necessary for this hazard.

15.4.4 Head and shell removal in combination with pre-export and on-arrival batch testing

When determining if head and shell removal combined with pre-export and on-arrival batch testing would reduce the overall risk of YHV1 to meet Australia's ALOP, the key points considered were:

- Sensitive qRT-PCR methods are available to detect YHV1 in prawns (Wijegoonawardane, Cowley & Walker 2010).
- Batch testing of prawns for YHV1 on-arrival in Australia (after the application of head and shell removal and pre-export testing) would reduce the likelihood of entry from low to extremely low. Under this scenario, testing and sampling is conducted under departmental control and oversight.
- Pre-export and on-arrival testing does not change the appearance of the prawns, therefore it is not considered to reduce the likelihood of them being used for unintended purposes (such as bait or berley), more than head and shell removal does. As such, the exposure likelihood with pre-export and on-arrival testing applied is consistent with the exposure likelihood of head and shell removal.

Conclusion

Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, head and shell removal in combination with pre-export and on-arrival batch testing, applied was determined to be **negligible.**

15.4.5 Cooking

When determining if cooking would reduce the overall risk of YHV1 to meet Australia's ALOP, the key points considered were:

- YHV1 is reported to be inactivated by heating at 60°C for 15–30 mins (Cowley et al. 2011; Flegel et al. 1995).
- YHV1-infected prawns cooked to reach a core temperature of 60°C, 70°C, 75°C, 85°C, and 95°C were not capable of transmitting YHV1 to healthy prawns following *per os* exposure (Aquaculture Pathology Laboratory & Department of Agriculture 2022a).
- Given the data about the effect of cooking on YHV1 infectivity, cooking to attain a core temperature of at least 65°C is therefore expected to significantly reduce the likelihood of entry of infectious YHV1 in imported prawns.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. Cooking is expected to further reduce the likelihood of this occurring because the nutritional benefits associated with this practice are removed through cooking. Cooked prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- Cooked prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). Therefore, cooking will also reduce the likelihood of prawns being used as bait or berley and consequently reduce exposure of wild crustaceans to imported prawns. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns (Moore et al. 2022). Consequently, it is assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels.

Conclusion
Based on this information, the overall restricted risk of imported frozen prawns with the biosecurity measure, cooking (with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C), applied was determined to be **negligible**.

15.4.6 Value-added products

When determining if uncooked prawns processed into a value-added product (VAP) would reduce the overall restricted risk of YHV1 to meet Australia's ALOP, the key points considered were:

- Value-added products are products in which the uncooked prawns have had the head and shell removed and have been further processed (see section 5.1.5 Value-added products). The likelihood of entry of YHV1 is expected to be the same as for head and shell removal. This is because it is not expected that the processing will further reduce the amount of viable YHV1 in the product more than head and shell removal does.
- Crustaceans in farms and hatcheries are considered unlikely to be deliberately exposed to whole uncooked prawns used as feed for maturation purposes as this behaviour is not practised in Australia. VAP would be even less likely to be used as a feed source because the nutritional benefits associated with this practice are removed through processing into a VAP. VAP prawns are also expected to be unattractive feed sources for crustaceans in research and public aquaria for the same reason.
- The likelihood of wild susceptible species being exposed to VAP would also be significantly reduced. Processed prawns are not a preferred bait source for recreational fishers in Australia (Moore et al. 2022). This may be due to several factors including the general higher cost of VAP and the lower percentage of prawns present in the product.

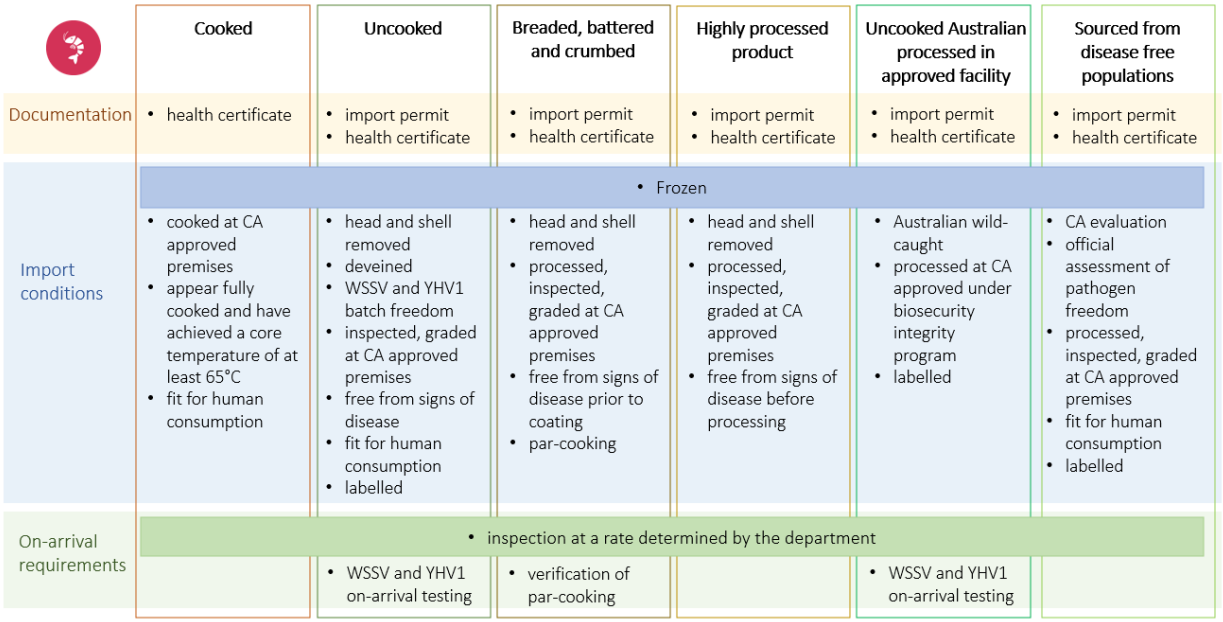
Conclusion
Based on this information, the overall restricted risk of imported frozen uncooked prawns which have been processed into a value-added product, was determined to be **negligible**.

16 Proposed biosecurity measures for imported prawns

The proposed import conditions for prawns and prawn products exported to Australia are provided in this chapter. A summary of the proposed import conditions in shown in Figure 7 and details are given in the body of the chapter. Details of the risk assessment values for determining how the biosecurity measures manage the biosecurity risk for each hazard to a level that meets Australia's appropriate level of protection (ALOP) are shown in Appendix F.

Those seeking to propose alternative biosecurity measures to those presented, should provide a submission to the department for consideration. Such proposals should include supporting scientific data that explain the extent to which the alternative measures would achieve Australia's ALOP. Biosecurity measures which require case-by-case assessment (for example, sourcing from disease free countries, zones or compartments) were not considered in detail for each hazard as part of this risk review.

Figure 7 Summary of the proposed import conditions for prawns and prawn products exported to Australia



WSSV white spot syndrome virus. **YHV1** yellow head virus genotype 1. **CA** the Veterinary Authority or other Government Authority of a Member Country having the responsibility and competence for ensuring or supervising the implementation of aquatic animal health and welfare measures, international health certification and other standards and recommendations in the WOAH Aquatic Code in the whole territory. **Labelled** prawns are in packages marked with the words "*For human consumption only. Not to be used as bait or feed for aquatic animals*".

The final import conditions will be developed based on the proposed biosecurity measures and published on the Australian Biosecurity Import Conditions (BICON) website.

16.1 General requirements

16.1.1 General certification requirements

Australia's certification requirements for prawns imported for human consumption align with international requirements (refer Chapter 5.1 and 5.2 of the World Organisation for Animal Health (WOAH, formerly OIE) *Aquatic animal health code* (WOAH Code)) and incorporate Australia's import conditions.

In line with certification standards and responsibilities defined in the WOAH Code, the department considers attestations by a country's Competent Authority (CA) to provide the most reliable document-level assurance attainable for each country. Industry assurances (such as manufacturer's declarations) are not relied upon for uncooked prawns.

Australia implements verification activities at the border to provide additional assurances that the certified conditions associated with each consignment are valid and meet biosecurity requirements. This process involves detailed document assessment and physical inspection of goods.

16.1.2 Verification, compliance and on-arrival inspection activities

The department applies risk-based inspection regimes to the different imported prawn categories. Non-routine interventions are a matter for the department's compliance and enforcement area and will consider information about importer behaviours and other intelligence. Other non-routine interventions undertaken by the department include retail testing and testing of imported prawns for emerging diseases (refer to section 17.3 Retail testing for white spot syndrome virus and section 17.4 Testing of imported uncooked prawns for emerging diseases). Trade volumes in each category are monitored and where unexpected increases are noted, additional attention is given to investigate potential for non-compliance. For example, import volumes of breaded, battered and crumbed prawns increased significantly following implementation of enhanced import conditions for uncooked prawns in July 2017, resulting in increased non-compliance in the breaded, battered and crumbed prawn pathway as a means to import uncooked prawns and avoid testing. The department monitored this change in import volume and along with intelligence gathering, determined and implemented a suitable adjustment to import conditions to manage the increased potential for biosecurity risk material to enter Australia.

On-arrival inspection activities are an operational means to ensure compliance with import conditions. Whilst the department currently implements seals intact inspections at various rates for imported prawns (as outlined in section 16.3 Import conditions), equivalent methods to ensure compliance may be considered by the department in the future, including systems such as the compliance-based intervention scheme. The implementation of equivalent biosecurity measures will be done at the discretion of the department following rigorous consideration of biosecurity risk.

The department applies routine and ongoing document and physical goods inspections and undertakes targeted interventions and time-limited verification activities as deemed necessary.

16.2 Documentation requirements

16.2.1 Existing import permit requirements

Cooked prawns
Cooked prawns and prawn products do not require an import permit providing they meet the requirements for cooked prawns.

Uncooked prawns
The importer must obtain a permit to import all uncooked prawns for human consumption into Australia. This applies to:

- uncooked prawns (prawns which have been deveined and had the head and shell removed (the last shell segment and tail fans permitted))
- uncooked breaded, battered or crumbed (BBC) prawns
- dumpling and dim sum-type products containing uncooked prawns
- uncooked wild-caught prawns of Australian origin processed overseas in approved premises
- prawns sourced from a country, zone or compartment that is recognised by Australia to be free of pathogenic agents of biosecurity concern.

The import permit must be obtained from the Department of Agriculture, Fisheries and Forestry before the goods are imported.

The application to import must include:

- the name and address of the importer and exporter
- a description of the commodity to be imported.

The application will be assessed on this information as well as any other criteria deemed relevant by the Delegate of the Director of Biosecurity.

16.2.2 Certification

Cooked prawns
Cooked prawns are required to meet conditions that are specified in the Biosecurity (Conditionally Non-prohibited Goods) Determination 2021 and must be accompanied by a certificate from a body listed in the List of Overseas Authorities—Aquatic Animals for Import (also known as the 'CA'[2]).

Uncooked prawns
Uncooked prawns must be accompanied by an official government health certificate from the CA in the exporting country.

16.3 Import conditions

Prawns and prawn products must meet one of the import conditions in this section, in addition to the permit (if applicable) and certification requirements, to be permitted import into Australia.

[2] The country CA is the Veterinary Authority or Government Authority accepted by the department to issue the certification required for all imported prawns and prawn products.

16.3.1 Cooked prawns

Product definition
Cooked prawns are prawns or prawn products which are frozen, appear fully cooked and have achieved a core temperature of at least 65°C during the cooking process.

Minimum cooking time is not specified for cooked prawns.

Certification requirements
The CA in the exporting country must certify on an official government health certificate that the cooked prawns:

a) are frozen and have been cooked in premises approved by and under the control of the CA and because of the cooking process the prawns appear fully cooked and have achieved a core temperature of at least 65°C.

b) are fit for human consumption.

On-arrival requirements
On arrival in Australia each batch of cooked prawns may be subject to inspection at a rate determined by the department. Alternative intervention methods or equivalent biosecurity measures may be considered by the department such as the implementation of a compliance-based intervention scheme.

16.3.2 Uncooked prawns

Product definition
Uncooked (also known as raw or green) prawns are prawns which have been deveined and had the head and shell removed (the last shell segment and tail fans permitted) and are frozen. Uncooked prawns may be marinated prawns, or Australian prawns processed overseas in facilities which have not been assessed and approved by the department through an official evaluation of the exporting country's CA.

Certification requirements
The CA in the exporting country must certify on an official government health certificate that the uncooked prawns:

a) are frozen and have had the head and shell removed (the last shell segment and tail fans permitted)

b) have been deveined (removal of the digestive tract to at least the last shell segment)

c) from each batch[3] has been found post-processing to be free of white spot syndrome virus (WSSV) and yellow head virus genotype 1 (YHV1) based on a sampling and testing method recognised by the WOAH for demonstrating absence of disease

[3] For the purposes of testing prawns for pathogenic agents of biosecurity concern, a batch may be defined (to be determined by the CA of the exporting country) as either:

1. product from a single line in a single processing run

d) have been inspected and graded in a premises approved by and under the control of the CA

e) are free from visible signs of infectious diseases

f) are fit for human consumption

g) are in packages marked with the words "*For human consumption only. Not to be used as bait or feed for aquatic animals*".

On-arrival requirements
On arrival in Australia each batch of uncooked prawns are subject to seals intact inspection and testing for WSSV and YHV1 at a rate determined by the department. Alternative intervention methods or equivalent biosecurity measures may be considered by the department, such as the implementation of the compliance-based intervention scheme.

16.3.3 Breaded, battered and crumbed prawns

Product definition
Breaded, battered and crumbed (BBC) prawns are uncooked prawns which have had the head and shell removed (the last shell segment and tail fans permitted), are coated for human consumption by being breaded, battered or crumbed, and have undergone a par-cooking step after the prawn has been coated, and are frozen.

Par-cooking is defined as the application of heat (for example, pre-frying, baking) after the prawn meat has been coated, to ensure the coating is set into a solid form and fully adheres to frozen and thawed prawns. There is no cooking time or temperature requirement for these product types.

Prawn products that do not meet all the import conditions outlined for BBC prawns will be subject to the import conditions for Uncooked prawns.

Certification requirements
The CA in the exporting country must certify on an official government health certificate that:

a) the BBC prawns are frozen, have been processed, inspected and graded in premises approved by and under the control of the CA

b) the prawns were free from visible signs of infectious diseases prior to coating

c) the BBC prawns have undergone a par-cooking step (for example, pre-frying[4] or baking) after the prawns have been coated to solidify and adhere the coating to the prawn.

2. product harvested from a single aquaculture pond (that is, prawns harvested from separate ponds are considered separate populations for the purposes of defining a batch)

3. one species of prawn wild-caught during one continuous fishing period.

Each consignment (container) will be considered as one batch unless multiple batches are specified in the container. If a batch is shipped in two containers, each container will be considered a single, unrelated batch. In addition, each batch in a consignment must be labelled and clearly identifiable.

[4] The Codex definition of pre-frying is: "Frying of breaded and battered fishery products in an oil bath in such a way that the core remains frozen" (Codex Alimentarius, Code of practice for fish and fishery products, CAC/RCP 52-2003).

On-arrival requirements
On arrival in Australia each batch of BBC prawns are subject to seals intact inspection and verification of the par-cooking step at a rate determined by the department. Alternative intervention methods or equivalent biosecurity measures may be considered by the department such as the implementation of a compliance-based intervention scheme.

16.3.4 Dumpling and dim sum-type products which contain uncooked prawns

Product definition
Dumpling and dim sum-type products are products which contain uncooked prawns (which have had the head and shell removed (the last shell segment and tail fans permitted)) which have been processed to the extent that no discernible pieces of meat are salvageable, and are frozen. They include all types of dumpling, spring roll, samosa, roll, ball or dim sum-type products (containing uncooked prawns).

Certification requirements
The CA in the exporting country must certify on an official government health certificate that the dumpling and dim sum-type products:

a) are frozen and have been processed, inspected and graded in premises approved by and under the control of the CA

b) the prawns were free from visible signs of infectious diseases before they were processed.

On-arrival requirements
On arrival in Australia each batch of dumpling and dim sum-type products are subject to inspection at a rate determined by the department. Alternative intervention methods or equivalent biosecurity measures may be considered by the department such as the implementation of a compliance-based intervention scheme.

16.3.5 Uncooked wild-caught prawns of Australian origin processed overseas in approved premises

Product definition
Uncooked wild-caught prawns of Australian origin must have been processed at a CA approved establishment, in accordance with the agreed biosecurity integrity program. For example, Thai Union Frozen Products Public Company Ltd has been approved by both the department and Thailand's CA to process Australian prawns for export to Australia.

Uncooked wild-caught prawns of Australian origin which do not meet all the import conditions outlined for 'uncooked wild-caught prawns of Australian origin processed overseas in approved premises' will be subject to the import conditions for Uncooked prawns.

Certification requirements
If assessed and approved by the department, the CA in the exporting country must certify on an official government health certificate that the uncooked prawns:

a) are frozen wild-caught prawns of Australian origin, processed at a CA approved establishment in accordance with the biosecurity integrity program agreed with the Department of Agriculture, Fisheries and Forestry

b) are in packages marked with the words "*For human consumption only. Not to be used as bait or feed for aquatic animals*".

On-arrival requirements

On arrival in Australia each batch of uncooked prawns are subject to seals intact inspection and testing for WSSV and YHV1 at a rate determined by the department. Alternative intervention methods or equivalent biosecurity measures may be considered by the department such as the implementation of the compliance-based intervention scheme.

16.3.6 Prawns sourced from a country, zone or compartment that is recognised by Australia to be free of pathogenic agents of biosecurity concern

Product definition

Prawns sourced from disease free countries, zones or compartments may be exported to Australia as frozen, uncooked whole prawns, partially peeled, peeled or other. To recognise this condition, the department would need to undertake an evaluation of the exporting country's CA to approve the trade.

Certification requirements

If assessed and approved by the department, the CA in the exporting country must certify on an official government health certificate that the prawns or prawn products:

a) are frozen and have been sourced from a country, compartment or zone that is recognised by Australia to be free of:
   i. "*Candidatus* Hepatobacter penaei" (only if the product is chilled)
   ii. covert mortality nodavirus
   iii. decapod iridescent virus 1
   iv. *Enterocytozoon hepatopenaei*
   v. infectious myonecrosis virus
   vi. Laem-Singh virus
   vii. Taura syndrome virus
   viii. *Vibrio parahaemolyticus* strains containing Pir toxins
   ix. white spot syndrome virus (WSSV)
   x. yellow head virus genotype 1 (YHV1).

b) have been processed, inspected and graded in premises approved by and under the control of the CA

c) are free from visible signs of infectious diseases

d) are in packages marked with the words "*For human consumption only. Not to be used as bait or feed for aquatic animals*".

On-arrival requirements

If uncooked prawns are sourced from a country, zone or compartment recognised by Australia to be free of the pathogenic agents, batch-testing for WSSV and YHV1 pre-export and on-arrival in Australia is not an import requirement. However, verification activities may be implemented at the border to provide Australia with ongoing assurances that trade in prawns from a country, zone or compartment achieves Australia's ALOP. Verification may include an appropriate level of on-arrival testing at a rate considered appropriate by the department for any of the pathogenic agents listed in Certification requirements.

16.4 Review of processes

16.4.1 Audit of protocol

The department may, at any time deemed necessary (and before commencement of trade), request information or seek to visit areas in exporting countries that produce prawns for export to Australia. The information requested and visits will be for the purposes of verifying the implementation of agreed import conditions and sanitary systems. These verification visits and audits may be undertaken in-person or remotely.

16.4.2 Review of policy

The department can review the import policy at any time.

16.5 Meeting Australia's food standards

Imported food for human consumption must satisfy Australia's food standards. Australian law requires that all food, including imported food, meet the standards set out in the Australia New Zealand Food Standards Code. Food Standards Australia New Zealand (FSANZ) is responsible for developing and maintaining the Food Standards Code, available on the Legislation website. The standards apply to all food in Australia, irrespective of whether it is grown domestically or imported.

17 Testing of imported prawns

Under enhanced import conditions implemented on 7 July 2017, all imported uncooked prawn consignments must be tested in the country of export post-processing, and again on arrival in Australia for white spot syndrome virus (WSSV) and yellow head virus genotype 1 (YHV1).

An improved inspection process, sampling regime and standardised testing protocol has also been implemented for consignments of imported uncooked prawns. Additionally, the department undertakes verification testing of imported uncooked prawns collected from Australian retail outlets to provide assurance that the enhanced import conditions are operating as expected.

17.1 Pre-export batch testing

Australia's import conditions for prawns require that each batch of uncooked prawns must be found, post-processing, to be free of WSSV and YHV1 based on sampling and testing methods recognised by the World Organisation for Animal Health (WOAH, formerly OIE) for demonstrating absence of disease.

Laboratories performing pre-export testing of uncooked prawns for Australia must be approved and recognised by the certifying competent authority, to apply diagnostic test methods aligned with those recommended by the WOAH. Health certificates accompanying consignments of uncooked prawns must include details of the pre-export testing laboratory and a testing report number. To achieve consistency in the sampling and testing procedures conducted in the exporting country and on-arrival in Australia, the department has provided the standardised testing procedure for WSSV to trading partners. The department recommends that trading partners implement the standardised procedure for pre-export testing to minimise the likelihood of inconsistency between pre-export and on-arrival test results for the same batch.

17.1.1 Engagement with exporting countries

The department provides engagement, education and support to competent authorities (CA) of exporting countries, to ensure they are aware and able to meet the import conditions. The department undertook familiarisation visits to prawn exporting countries in 2017 and 2018 to observe pre-border controls for prawns, including pre-export sampling and testing, and provided feedback for improvements as necessary.

The department also funds the Asia-Pacific Laboratory Proficiency Testing (PT) Program on Aquatic Animal Diseases, a four-year program (2018–22) designed to strengthen laboratory diagnostic capabilities for aquatic animal diseases of significance across the Asia-Pacific, the region that exports most uncooked prawns to Australia. The department also funded a regional PT program in 2013–15 that saw an improvement in the regional capability of Australia's trading partners to detect and diagnose important aquatic animal diseases. The PT program was developed in collaboration with the Australian Centre for Disease Preparedness (ACDP) and the Network of Aquaculture Centres in Asia Pacific (NACA) and contains 10 pathogens, including WSSV and YHV1. There are currently 39 laboratories from within the Asia-Pacific region participating in the PT program, some of which conduct pre-export testing of uncooked prawns imported to Australia.

17.1.2 Batch definition

For the purposes of testing prawns for pathogenic agents of biosecurity concern, a batch may be defined (to be determined by the CA of the exporting country) as either:

1) product from a single line in a single processing run
2) product harvested from a single aquaculture pond (that is, prawns harvested from separate ponds are considered separate populations for the purposes of defining a batch)
3) one species of prawn wild-caught during one continuous fishing period.

Each consignment (container) will be considered as one batch unless multiple batches are specified in the container. If a batch is shipped in two containers, each container will be considered a single, unrelated batch. In addition, each batch in a consignment must be labelled and clearly identifiable.

Documentation verifying the number of batches in the consignment must be provided to the department. This documentation must clearly detail the labelling of each batch in the consignment. If the number of batches cannot be determined from documentation, full unpacking and inspection may be required to determine the number of batches. This may result in additional testing and inspection costs.

The department will review batch definitions and evidence required to support batch identification and product traceability for imported uncooked prawns once the Australian National University's imported prawn sampling program review has concluded (refer to section 17.2.2 Sampling design).

17.2 On-arrival batch testing

Australia's import conditions for prawns require that each batch of uncooked prawns must be found on-arrival to be free of WSSV and YHV1 using the inspection, sampling and testing procedures described in this section.

17.2.1 Inspection

Under the current implementation of the on-arrival batch testing for uncooked prawns, shipping containers must remain unopened and held seals-intact under biosecurity control at an Approved Arrangement (AA) site approved to handle imported uncooked prawns until a biosecurity officer is present. All uncooked prawn inspections are conducted by two biosecurity officers. Biosecurity officers verify the importer's commercial documentation and seal integrity prior to the inspection commencing. The container seal can only be broken in the presence of a biosecurity officer and is followed by a full supervised unpack of the prawn consignment. All consignments are held under biosecurity control while awaiting the results of testing.

In addition to the sampling for WSSV and YHV1 testing, biosecurity officers randomly select and inspect 10% of the goods, including the external and internal markings of the cartons, to ensure they match described packaging information and documentation.

Alternative intervention methods or equivalent biosecurity measures may be considered and implemented by the department at any time. These could include equivalent measures such as pre-export testing programs or the compliance-based intervention scheme. If these measures were to be implemented, rates of intervention would be determined by the department based on the specifics of the equivalence measures and following rigorous assessment of the biosecurity risks.

17.2.2 Sampling design

The imported prawn sampling program is designed to provide 95% confidence of pathogen detection within a batch if present at a prevalence of 5% or greater. The sampling design parameters are in accordance with those recommended in the WOAH *Aquatic animal health code* (WOAH Code) (WOAH 2022c). Chapter 1.4 of the WOAH Code states when designing a surveillance programme to demonstrate freedom from disease, the required level of confidence in the surveillance system (probability that the system would detect infection, if infection were present at the specified level) should be greater than or equal to 95% (OIE 2021c). The WOAH Code further states a suitable design prevalence value at the animal level may be over 5% for highly transmissible infections (WOAH 2022c). Both WSSV and YHV1 would be considered highly transmissible based on the available scientific literature. That is, if WSSV and YHV1 were present in a farmed prawn population, the true prevalence is likely to be much greater than 5% (OIE 2021k, l). Prevalence of WSSV and YHV1 in wild prawn populations is also expected to be greater than 5% (Hamano et al. 2017; Muhammad et al. 2020; Xu et al. 2021b).

The sample design is based on a large population (batch) size and considers that a batch may be comprised of prawns from more than one source (that is, some heterogeneity is accepted). The sample size is determined by the large population size, the desired confidence and pathogenic agent prevalence. Since July 2017, the average weight for a single batch of uncooked prawns has been approximately 12 tonnes. If we assume an average peeled, de-headed prawn size of 10g this equates to 1,180,000 prawns/batch. It should be noted that while the average batch sizes have increased between 2009 and 2021 (3.5 tonne in 2009 and 12 tonnes in 2021), both populations are large and the sample size required to achieve 95% confidence of pathogen detection at 5% prevalence remains the same (Sergeant 2021). The sample size represents a risk-managed and practical approach to achieving the required level of confidence to achieve Australia's appropriate level of protection (ALOP). For example, at present, 65 prawns sampled across 13 randomly selected boxes (with 5 prawns pooled per test) achieves that level.

It should be noted that a sampling protocol using a 5% design prevalence to achieve 95% confidence does not necessarily mean that the hazard would go undetected if less than 5% of prawns in a consignment contained the hazard. Where the true prevalence of a hazard within a consignment drops below 5%, the sampling protocol still has a chance of detecting the hazard, but the confidence level (that is, the probability of detecting the hazard) is reduced to below 95%. For example, the sampling design considers that if the true prevalence was 2.5% then the confidence level for detecting the pathogen is 79%. It should also be noted that as the true prevalence drops, so does the biosecurity risk.

The original sample design assumed a diagnostic test sensitivity of 95% (consistent with the published information available of diagnostic methods) and considered tissue pooling (five prawns) to have no impact on diagnostic sensitivity. The department commissioned ACDP to provide a formal validation report for the WSSV qPCR and report on any impacts on test sensitivity through pooling. ACDP's validation manuscript was recently published (Moody et al. 2022) and the results of this work are being considered in the context of the imported prawn sampling design.

Since 1999, the department has sought advice from external and internal expert statisticians to inform the sampling design. The department has consistently applied the advice received when

implementing the sampling program. In line with the release of the draft report, the department has again engaged with statistical experts within Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) and an independent statistical expert at the Australian National University to review the imported prawn sampling design, with a focus on the increased batch sizes and the potential implications for larger batches to be comprised of prawns from greater numbers of epidemiologically distinct source populations. The department will consider outcomes of this review and make any necessary adjustments to the sampling program design to maintain the desired level of confidence. This will include reviewing batch definitions, sample sizes and evidence required to support batch identification and product traceability for imported uncooked prawns.

17.2.3 On-arrival sampling procedures

Samples of uncooked prawns for WSSV and YHV1 testing are taken by biosecurity officers following prescribed procedures that ensure consistent and representative sampling. Prawns are sampled from randomly selected cartons from each importer-declared batch. The number of prawns taken from each batch should provide a 95% level of confidence of detecting the hazard, if present, at a prevalence of 5% or greater in the batch. For example, 65 prawns sampled across 13 randomly selected boxes currently achieves that level of confidence. Samples are then packaged according to defined procedures and submitted to an approved screening laboratory for testing.

17.2.4 Approved Australian screening laboratories

Only department-approved Australian screening laboratories are permitted to undertake on-arrival testing for WSSV and YHV1. A program to strengthen laboratory testing requirements for WSSV was implemented in 2017. Through this program, the department has implemented a standardised testing procedure for WSSV (refer section 17.2.5 Testing procedures). The procedure is prescribed for use by approved Australian screening laboratories testing imported prawns on-arrival with the objective to ensure consistency in methodology and results interpretation. The department also coordinates with ACDP, a quality assurance program (QAP) for WSSV testing at approved Australian screening laboratories. The QAP is designed to monitor each laboratory's ongoing proficiency in using the standardised testing procedure for WSSV. Participation in the QAP is mandatory for approved Australian screening laboratories. There are three rounds of the QAP each year. To date, all approved Australian screening laboratories have demonstrated acceptable results. The department has added YHV1 to the QAP and may incorporate additional pathogens as necessary in the future to align with import conditions.

The department has developed a new AA class for Australian screening laboratories approved by the department for testing imported prawns. The new AA class was approved for implementation in September 2020 to facilitate improved regulation of Australian screening laboratories undertaking imported prawn testing. Australian screening laboratories will be assessed by the department under the new AA Class 15.1 conditions following the outcomes of ACDP's formal validation of WSSV qPCR methods and each laboratory's re-accreditation by the National Association of Testing Authorities (NATA). ACDP's manuscript of the formal validation of two WSSV qPCR methods was recently published and confirmed that the assays have comparable performance characteristics with acceptable repeatability, diagnostic sensitivity, and specificity for use on sub-clinically and clinically infected prawns (Moody et al. 2022). This information will be provided to approved Australian screening laboratories. NATA will re-assess each approved laboratory against the International Organization for Standardisation/International Electrotechnical Commission (ISO/IEC) 17025:2017

standard and verify their competence to implement the standardised WSSV testing procedure and achieve performance criteria specified in the validation manuscript. All approved Australian screening laboratories are currently ISO/IEC 17025 accredited by NATA.

17.2.5 Testing procedures

The testing methods currently applied by approved Australian screening laboratories for detection of WSSV and YHV1 are based on methods listed in the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH Manual) (WOAH 2022l) or equivalent.

White spot syndrome virus

The WSSV testing procedure uses 5 prawns pooled as one sample, with each pooled sample represented by a single test result. A standardised WSSV testing procedure to strengthen consistency of on-arrival virus testing for uncooked prawns was implemented at all approved Australian screening laboratories in October 2017. This procedure was developed in partnership with the approved Australian screening laboratories, ACDP and Australia's national laboratory accreditation body, NATA. This procedure provides TaqMan qPCR methods for the detection of WSSV DNA for biosecurity risk management. Two TaqMan qPCR methods for WSSV are described in this procedure and they are both recognised by the Australian Government Department of Agriculture, Fisheries and Forestry as suitable for purpose. The two methods are based on the TaqMan qPCR (Durand & Lightner 2002; OIE 2021k) outlined in WOAH Manual and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) TaqMan qPCR (Sritunyalucksana et al. 2006b). The key difference between the standardised WSSV testing procedure and the WSSV Taqman qPCR methods described by Durand & Lightner (2002) and Sritunyalucksana et al. (2006) relate to PCR cycling times and criteria for results interpretation. The standardised procedure is run for 45 cycles (not 40) and any amplification curves passing the cycle threshold (Ct) up to 40 cycles are considered a positive result. Any amplification curves passing the cycle threshold between 40 and 45 cycles are considered a suspect positive result. Suspect positive samples are considered 'positive' unless confirmatory re-testing at ACDP has found them to be negative. A negative result occurs when there are no amplification curves crossing the threshold within 45 cycles in all samples.

ACDP recently published a manuscript of the formal validation of the two TaqMan qPCR methods for WSSV (Moody et al. 2022). ACDP demonstrated that both assays have comparable performance characteristics, with acceptable repeatability, diagnostic sensitivity (DSe) and specificity for use on sub-clinically and clinically infected prawns (Moody et al. 2022). The CSIRO WSSV qPCR had slightly higher DSe and was statistically more repeatable, compared to the WOAH WSSV qPCR (Moody et al. 2022). Therefore, at ACDP, the CSIRO WSSV qPCR is considered the primary screening assay, with the WOAH WSSV qPCR considered an acceptable alternate assay.(Moody et al. 2022). The imported prawn sampling program allows for both assays as a primary screening assay.

Yellow head virus genotype 1

Approved Australian screening laboratories implement procedures equivalent to those listed in the WOAH Manual (WOAH 2022l) for testing uncooked prawns for YHV1. The YHV1 testing procedures also uses 5 prawns pooled as one sample, with each pooled sample represented by a single test result. The methods target YHV1-specific regions of the genome and are based on reverse transcriptase real-time PCR (RTqPCR) methods. ACDP is the WOAH reference laboratory for yellow head viruses and is currently undertaking a project to develop and validate diagnostic methods for detection of YHV1 and differentiation from others genotypes in the yellow head complex. The

outcomes of this work will be considered in the context of testing protocols applied on-arrival for YHV1.

17.2.6 Test result outcomes

Once a negative test result is received, the batch may be released from biosecurity control. If a batch returns a positive test result for either WSSV or YHV1 via screening testing, it is not released from biosecurity control. Importers have the option to request a confirmatory test on the positive samples through ACDP or to accept the results of screening testing. All results from confirmatory testing are considered final, including where confirmatory testing may return a different result to screening testing. If any of the samples are concluded to be WSSV or YHV1-positive, the entire batch must be exported, treated (cooked) or destroyed.

For those batches that return a positive test result, the department will notify the exporting country's CA to maintain their awareness and encourage ongoing improvements to reduce future incidences of WSSV or YHV1-positive product being exported to Australia. In some cases, depending on the nature of the positive detection, the department will request a CA to investigate the cause of the positive batch, and implement appropriate corrective actions to address any identified issues.

On-arrival testing results for white spot syndrome virus

Since the resumption of trade in uncooked prawns in July 2017, 1.17% (51/4,338) of prawn consignments have tested positive on-arrival for WSSV (as of 31 December 2022).

For the 2020–21 year, 1.19% of prawn consignments (10/838) tested positive for WSSV on-arrival. For the 2021–22 year, 0.59% of prawn consignments (6/1,022) tested positive for WSSV on-arrival. For the 2022–23 (current to 31 December 2022), 1.29% of prawn consignments (7/540) have tested positive for WSSV on-arrival.

17.3 Retail testing for white spot syndrome virus

The department undertakes a range of activities to verify that Australia's current import conditions for prawns are being met, and to provide assurance that ongoing trade achieves Australia's ALOP. One of these activities is WSSV testing of imported prawns from Australian retail outlets Under the department's retail sampling program, officers purchase sealed bags where an importer and batch code can be identified to enable traceback. Three samples of five prawns are then taken from each bag (15 prawns total) and each pool of five is tested for WSSV. Each bag is therefore tested three times.

- In 2017, 14 out of 19 prawn samples collected from retail outlets in the Logan River area, Queensland returned positive results for WSSV.
- In 2018, 101 imported uncooked prawn products were collected from various Australian retail outlets and tested for WSSV.
  - Two samples collected from Melbourne returned a strong positive result and 4 other samples (from Sydney and Melbourne) returned a weak positive result.
  - The 2 positive samples were traced back to the same importer who elected to dispose of the remaining 40 cartons of product that were in storage.
  - These were linked to an exporter and importers that are no longer permitted to trade prawns with Australia.

- Retail testing conducted in July 2019 found no evidence of WSSV in the 38 imported prawn samples tested.
- Over the past two rounds in June 2021 and 2022, 363 samples were sent to ACDP for testing.
  - A low-level detection of WSSV occurred from a bag of prawns purchased in a retail shop in Sydney in June 2022.
  - This is the first WSSV detection in retail prawns since 2018.
  - In this instance, only one of the three samples taken from the relevant bag returned a positive result at ACDP for WSSV.
  - The department conducted a risk assessment and determined that for this one batch, taking into account entry and exposure likelihoods, the biosecurity risk did not exceed Australia's ALOP.

17.4 Testing of imported uncooked prawns for emerging diseases

The department investigates the presence of emerging pathogenic agents in imported prawns by testing of batches of imported uncooked prawns. Batches of uncooked prawns that have been tested for WSSV and YHV1, with negative results, are sampled in this program. Australian-origin prawns processed overseas are excluded.

The results obtained from this program are not used by the department to make regulatory decisions on individual batches of imported prawns. The prawn batches are released from biosecurity control prior to testing for the emerging pathogenic agents. Trading partners have access to their country specific information should they request it, however, no country specific data is publicly available. At this point, the cost is borne by the department.

The department has determined a statistically relevant sampling and testing regime for the program. It has also determined the frequency and whether specific countries should be targeted depending upon the pathogenic agents being investigated. To ensure the integrity of current pre-export and on-arrival testing programs, only emerging pathogenic agents which are not subject to pre-export and on-arrival testing are considered. Other verification activities for those pathogens subject to pre-export and on-arrival testing are undertaken already (refer section 17.3 Retail testing for white spot syndrome virus).

17.4.1 Investigation of batches of imported uncooked prawns for emerging diseases

In 2020–22, the department engaged ACDP to test randomly selected batches of imported uncooked prawns to monitor for the presence of covert mortality nodavirus (CMNV), decapod iridescent virus 1 (DIV1) and *Enterocytozoon hepatopenaei* (EHP). These hazards were selected as the department considered them emerging pathogens for which there was a lack of information about their presence in imported prawns and their geographical distribution. The program provided information that was used when considering the biosecurity risks for CMNV, DIV1 and EHP.

Design

During 2020-21, prawn samples already collected by the department for WSSV and YHV1 testing were tested by ACDP for CMNV, DIV1 and EHP. The program tested 90 batches of uncooked prawns. This represented over 10% of all batches imported into Australia in 2020–21 and covered the spectrum of countries exporting uncooked prawns to Australia.

An additional, more targeted, program was conducted in early-2022 which tested for CMNV and DIV1 in uncooked prawns exported from countries known to have endemic CMNV and DIV1. The program tested 8 batches of uncooked prawns, which represented over 80% of incoming consignments, from those countries, in that period of 2022.

Testing procedures
Imported batches were tested using prawn samples collected by departmental Biosecurity Officers (65 prawns per batch, as 13 pools of 5 prawns). These samples were collected from batches of imported prawns under secure seals intact conditions at approved arrangement locations for WSSV and YHV1 testing. Following testing, and negative results, for WSSV and YHV1, the remaining prawn tissue was transported to the ACDP for testing of CMNV, DIV1 and EHP at the department's direction, discretion and cost.

Testing procedures were based on published real-time PCR methods for CMNV (Pooljun et al. 2016), DIV1 (Qiu et al. 2018a; Qiu et al. 2020b) and EHP (Liu et al. 2018c).

Test results
All 90 batches of uncooked prawns tested negative for CMNV and DIV1. In the more targeted testing, none of the batches (0/8) from CMNV and DIV1 endemic countries tested positive for CMNV or DIV1.

EHP was detected in 66/90 (73.3%) batches. EHP positive results were confirmed via sequencing to ensure positives were EHP and not a false-positive caused by a similar microsporidian. The presence of EHP in these samples was not unexpected given the prevalence of EHP in exporting countries and given that the final tail segment of the prawn was selected for tissue collection, so that any potential faecal/intestine material would be present in each sample. However, knowing the impact of freezing on EHP infectivity, and that removal of the head and shell and deveining of the prawn also contribute to managing risk, the department does not consider that this result is cause for concern. Also noting that these PCR results determine the presence, not the viability of EHP.

17.4.2 Ongoing monitoring of batches of imported uncooked prawns

Ongoing monitoring of batches of imported uncooked prawns is an extension of the program outlined in section 17.4.1 Investigation of batches of imported uncooked prawns for emerging diseases.

Ongoing monitoring of batches of imported uncooked prawns for CMNV and DIV1 will enable the department to monitor changes and accurately determine the entry likelihood of these emerging diseases in imported prawns. Other hazards (for example "*Candidatus* Hepatobacter penaei", infectious myonecrosis virus, Laem-Singh virus, Taura syndrome virus, *Vibrio parahaemolyticus* strains containing Pir toxins and yellow head virus genotype 8), or new and emerging pathogenic agents (such as those listed in Table 6) of concern may also be included in this ongoing program on an as needed basis.

This program meets the recommendation by the members of the scientific advisory group that the final report include a design and implementation plan for the continuation of a random batch testing program to monitor for exotic diseases (refer Appendix A: Expert panel recommendations).

Design
Prawn samples will be tested for the hazards or emerging pathogenic agents of concern. Representative samples will be taken from prawns that are collected by the department for WSSV and YHV1 testing and received negative test results. This may be targeted based on specific source countries, or a survey of all countries may be necessary.

Testing procedures
Testing methods used for this program are based on published real-time PCR methods or WOAH recommended methods, where available. It is noted that such a program can only be implemented if there is a published testing method available for the pathogenic agent of interest. To continue improving the effectiveness of biosecurity measures, the department may specify alternative methods with higher diagnostic sensitivity and/or specificity than the methods recommended by the WOAH, as new methods become available.

Results
If the department determines that the results of this program demonstrate a change in the entry likelihood of the pathogenic agents, the department will consider whether other biosecurity measures, such as 100% pre-export and/or on-arrival batch testing (as outlined in section 5.1.8 Batch testing for hazards), are necessary to achieve Australia's ALOP.

Appendix A: Expert panel recommendations

The members of the scientific advisory group made nine recommendations and several suggestions for the department to consider when preparing the provisional final report.

It was recommended the provisional final report:

1) Addresses the top-level issues raised by the submissions to the 2020 draft report.
2) Provides more detail on the pre-export and on-arrival testing programs, and the associated monitoring and compliance program.
3) Gives a clearer account of the potential economic impacts of disease introduction to Australia.
4) Emphasises that the 'very low' risk rating for white spot syndrome virus does not assume that recreational fishers will comply with the voluntary code not to use imported prawns for bait or berley.
5) Includes the design and implementation plan for a random batch testing program to monitor for other exotic diseases (covert mortality nodavirus, decapod iridescent virus 1, *Enterocytozoon hepatopenaei*, infectious myonecrosis and Taura syndrome virus) in prawns imported for human consumption.
6) Outlines the department's extensive actions undertaken since the 2016 incursion, including its response to the recommendations of the Inspector-General of Biosecurity Uncooked prawn imports: effectiveness of biosecurity controls report 2017, and future planned activities.
7) Acknowledges that, on the balance of probabilities, the use of imported prawns for bait and berley by recreational fishers is the likely pathway of the 2016 disease incursion.
8) Indicates how the biosecurity arrangements for imported prawns are consistent with that taken for other imported meat products (beef, pork and chicken).

It was recommended the department:

9) Ensures that the biosecurity measures given in the draft report are properly resourced and monitored.

In their report on the provisional final report, the members of the scientific advisory group noted that:

1) Careful revision of sections 16.2 and 16.3 should occur to ensure stakeholder understanding of the proposed biosecurity requirements.
2) A small number of minor issues remained for the department to consider when preparing the final report. In summary, it was recommended that:
    a) The comment in column 3, Appendix B, be adjusted to be consistent with other parts of the report.
    b) Chapter 14 be updated considering the information provided in chapter 1.5.
    c) Section 5.1.3 be reworded to ensure clarity and consistency with other related sections of the report and text alluding to changes in labelling be strengthened (section 5.1.9).

d) The department consider making specific reference to the other eight diseases (section 17.4.2 Ongoing monitoring of batched of imported prawns uncooked prawns).

e) Additional text be included to indicate South Australia has an annual wild catch of approximately 2,000 tons (section 1.3).

f) Appendix F (table 26) be referenced in the text immediately above Table 1.

g) The department reword sections 14.4.3 and 14.4.4 to ensure clarity.

h) The department consider if the reference in Appendix B, issue 38, should be 'wild Australian prawns processed overseas in a departmental approved facility'.

Appendix B: Key issues raised by stakeholders

The department issued Animal Biosecurity Advice 2020-A05 on 28 September 2020, notifying stakeholders of the release of the *Review of the biosecurity risks of prawns imported from all countries for human consumption* – draft report. Seventeen submissions were received, and consent was given to publish 12 on the prawn review webpage. All stakeholder submissions were considered when preparing this final report.

The key issues raised by stakeholders about the draft report, the department's response to them and how this final report has been amended are summarised in Table 22.

Table 22 key issues raised by stakeholders and the departmental response to them

Issue number	Issue	Department response	Final report reference
-	General issues	-	-
1	Import risk assessments need to be updated in a timely manner, the current process is slow and unresponsive to changes in biosecurity risk, including emerging diseases.	**Noted**. A risk review is a very complex scientific process which takes time to conduct. The department constantly monitors ongoing media and scientific literature feeds about biosecurity issues for all animal species, those feeds are reviewed by technical experts, and it is anticipated that information about any reported emerging disease would be picked up through these information sources. Under the World Organisation for Animal Health (WOAH, formerly OIE), Member Countries are required to notify of the occurrence of listed diseases and emerging disease events. The department also uses resources such as the Network of Aquaculture Centres in Asia-Pacific (NACA) and communicates with our counterparts in other countries. If an alarm is raised through any of these processes regarding pathogenic agents of biosecurity concern, the department will review the risk and take immediate action if it is considered that the risk exceeds Australia's appropriate level of protection (ALOP). For example, implementation of the enhanced import conditions for prawns and prawn products since the resumption of trade in 2017. It is important to note that these reviews are most often not a formal risk review, and therefore they are only released publicly if the review determines changes need to be made.	3.1 Emerging diseases

Issue number	Issue	Department response	Final report reference
		An explanation about how the department responds to changes in biosecurity risk has been included in the final report.	
2	The Inspector-General of Biosecurity (IGB) review should have been addressed in the draft report.	**Noted and agreed.** The IGB undertook a review of the effectiveness of biosecurity controls implemented by the department for uncooked prawn imports. The IGB review contained 22 recommendations. Implementation of the 22 recommendations is near completion, supported by ongoing business as usual activity, with some requiring decisions by third parties. A summary section regarding the IGB review and an appendix detailing the progress against each recommendation has been included in the final report.	1.5.3 Inspector-General of Biosecurity review Appendix C Recommendations of the IGB's review of uncooked prawn imports and progress on their implementation
3	Trade concerns outside the prawn industry are the driver of final policy decisions and do little to reduce the biosecurity risk.	**Noted.** This risk review was conducted using a risk analysis process that is consistent with that described by the World Organisation for Animal Health (WOAH, formerly OIE) *Aquatic animal health code* (WOAH Code). The biosecurity measures recommended are based on science and whether they manage risk to a level that achieves Australia's ALOP, are practical and meet our rights and obligations under World Trade Organization (WTO) *Agreement on the application of sanitary and phytosanitary measures* (the SPS agreement). All WTO member countries are bound by the SPS agreement. The right to adopt sanitary and phytosanitary (SPS) measures is accompanied by obligations aimed at minimising negative impacts of SPS measures on international trade. The basic obligations are that SPS measures must: • Be applied only to the extent necessary to protect human, animal or plant life or health and not be more trade restrictive than necessary. • Be based on scientific principles and not maintained without sufficient scientific evidence. • Not constitute arbitrary or unjustifiable treatment or a disguised restriction on international trade.	No changes in final report.

Issue number	Issue	Department response	Final report reference
		Further information about the application of the SPS agreement is available on the department's website.	
-	**The national recreational fishing survey**	-	-
4	The bait and berley pathway risk cannot be properly assessed until the completion and release of the *National social and economic recreational fishing survey*.	**Noted and agreed**. The outcomes of the bait and berley component of the *National social and economic recreational fishing survey* are an integral part of the exposure assessments and are also considered when estimating the effectiveness of certain risk management measures. The department has considered and included the data from the bait and berley component of the *2019–20 National social and economic recreational fishing survey* in this final report.	1.6 Use of prawns as bait and berley by recreational fishers Appendix D, Use of imported prawns as bait or berley for recreational fishing
-	**Australia's biosecurity policy framework**	-	-
5	Australia's ALOP and risk management measures are not applied in a consistent way across aquatic and terrestrial products.	**Noted.** All WTO members are required to meet and uphold the principles and obligations of the SPS agreement. The SPS agreement provides WTO members with the right to use SPS measures to protect human, animal or plant life or health. Each WTO member is entitled to maintain a level of protection it considers appropriate to protect human, animal or plant life or health within its territory. This is called the ALOP. As per our international obligations, Australia applies ALOP in a consistent way across all products (that is, aquatic animal, terrestrial animal, and plant products). As unique risk factors and scenarios apply to each product, those risks are managed in different ways to ensure that Australia's ALOP is met. They also consider the WOAH recommendations for managing biosecurity risk associated with the product. Importantly, biosecurity measures are selected based on whether they reduce risk to a level that achieves Australia's ALOP. In the case of prawns, Australia's biosecurity measures are more stringent than those recommended by the WOAH, this is because they are supported by a scientific risk assessment. A paragraph explaining this issue has been included in the final report.	1.1.1 Australia's appropriate level of protection
-	**Hazard identification**	-	-

Issue number	Issue	Department response	Final report reference
6	During the hazard identification process, some pathogens were eliminated from further consideration based on a lack of evidence or based on the incorrect statement that there is insufficient evidence that the disease will be associated with imported adult prawns. This comment mostly refers to diseases such as hepatopancreas and digestive tract necrosis virus (HINV), *Macrobrachium rosenbergii* Golda virus (MrGV) and *Macrobrachium rosenbergii* Taihu virus (MrTV).	**Noted.** No changes were made to Table 5 in with respect to entries for *Macrobrachium rosenbergii* Golda virus (MrGV) and *Macrobrachium rosenbergii* Taihu virus (MrTV). The entry for HINV has been deleted as HINV is no longer considered to be the causal agent of glass post-larvae. Later studies identified *Vibrio parahaemolyticus* strains causing glass post-larvae as the causal agent of the disease, this has been added to the hazard table. Further review of all available information since the release of the draft report has supported the assumption that these pathogenic agents are unlikely to be associated with disease in commodity size prawns. The department will continue to monitor the situation with respect to these pathogenic agents.	3.1 Emerging diseases Table 5
7	The conclusions that the hazards identified in the draft report do not exist in Australia lacks evidence.	**Noted.** Australia has a long history of passive surveillance and a strong system in place to detect incursions of exotic diseases. The department is confident that the hazards in this final report are not present in Australia, except for white spot syndrome virus (WSSV). WSSV is limited to an area in South-East Queensland which is under an official control program. WSSV has also been detected at prawn farms on the Clarence Valley, NSW north coast. The farms were issued with a biosecurity direction by NSW Department of Primary Industries (DPI) to carry out destruction of affected animals and decontamination of the premises. Surveillance is being conducted to determine if the virus has been eradicated from the farms and to determine if the virus is present in the immediate environment in the vicinity of the farms. At the time of preparing this report, there is no indication that the virus has spread beyond the affected premises. A control order is in place which restricts the movement of raw, uncooked decapod crustaceans from the Clarence Estuary while containment, source detection and surveillance activities are underway. Australia's current import conditions will remain in place while WSSV is under official control. The process for finalising the prawn review will also continue under established procedures. This is consistent with our international rights and obligations.	No changes in the final report.

Issue number	Issue	Department response	Final report reference
		This is consistent with our international rights and obligations.	
-	**Risk assessment methodology and summary of general considerations**	**-**	-
8	Considering risk on an annual basis underestimates risk.	**Noted.** The department normally considers the likelihood of entry based on the estimated volume of one year's trade. Twelve months is considered a sufficient period to enable evaluation of seasonal effects, but not so long as to incorporate inaccuracies that may be associated with changes in disease factors, animal factors or factors associated with trade. Additionally, it would be difficult to investigate and to transparently or consistently demonstrate the effect that projected volume of trade may have on the risks associated with the importation of prawns. Because impacts may occur over an extended period, consideration of them is not limited to what might occur for one year but covers the period the impacts are discernible. This is considered when estimating the direct and indirect impacts as part of the consequence assessment. If there are substantial changes in the volume and nature of the trade in specific commodities then the department will review the risk analysis and, if necessary, provide updated policy advice.	No changes in the final report.
9	Sufficient weight of consequence has not been considered with respect to the incursion of WSSV, or any other exotic prawn disease, into the fisheries that supply broodstock to the domestic prawn farming industry.	**Noted**. The consequence assessment for the retained hazards has been reviewed and the estimations were considered appropriate. The potential adverse impacts of establishment and spread were evaluated against several criteria including the life or health of domestic or wild animals. This includes the impact on fisheries that supply broodstock to the domestic prawn farming industry. The impact score given to pathogens Vp AHPND (*Vibrio parahaemolyticus* strains containing Pir toxins), WSSV and YHV1 (yellow head virus genotype 1) was **F** (**significant impact at the national level**). Risk assessment outcomes are consistent with the current broodstock testing requirements in the jurisdictions. For example, the requirements for translocating prawn broodstock to New South Wales	No changes to the final report.

Issue number	Issue	Department response	Final report reference
		include PCR testing for AHPND-like disease, WSSV, YHV1 and yellow head virus genotype 7.	
10	The apparent 'sensitivity' of the analysis outcome (biosecurity recommendations) to the partial likelihood of establishment and spread (PLES) for wild crustaceans should be examined.	**Noted.** The PLES is combined with the overall impacts using the *Matrix for determining the likely consequences for the outbreak scenario* to determine likely consequences. The matrix is not symmetrical; the overall impacts are weighted more heavily. For example, a **Low** PLES combined with **High** overall impact gives a likely consequence rating of **Moderate**; this is not the same as a **High** PLES combined with **Low** overall impact, which gives a likely consequence rating of **Low**. Therefore, the PLES is not the most influential factor in the analysis.	No changes to the final report.
11	The assessments for partial likelihood exposure (PLE) and PLES for the exposure group "wild crustaceans" need to be reviewed as the following statement in the review is not supported: "*Wild crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley*."	**Noted and agreed.** The risk assessments for all hazards have been reviewed. It is considered that wild crustaceans are less abundant when compared to crustaceans in aquaculture facilities and that they may therefore encounter greater competition from other aquatic animals for prawn material in their environment. However, it is also acknowledged that regular introduction of prawn material into the aquatic environment through use as bait or berley occurs and that wild crustaceans are moderately likely to encounter this material. The wider the host range of the hazard, the higher the likelihood of wild susceptible crustaceans encountering the hazard, and the risk assessments for each hazard take these considerations into account. It is agreed that some wild crustaceans, such as schools of prawns may be at similar densities to those in farms. However, the water movement, predation from non-susceptible species and the general likelihood of schools of prawns encountering imported prawns if introduced into the wild are different to that in a farm or hatchery environment. The PLE and the PLES of decapod iridescent virus 1 (DIV1) in wild crustaceans has been increased. This is due to new information indicating a broader host range under natural conditions. The PLES of *Enterocytozoon hepatopenaei* (EHP) in wild crustaceans has been increased. This is due to new information indicating a broader	8 Decapod iridescent virus 1 risk review 9 Enterocytozoon hepatopenaei risk review 14 White spot syndrome virus risk review

Issue number	Issue	Department response	Final report reference
		host range, including additional vectors and carriers which are present in Australia, and EHP ability to remain infectious in infected prawns and the environment for extended periods. The PLES of WSSV in wild crustaceans was reconsidered and increased due to its broad host range and transmissible through water, the establishment and spread of WSSV in the directly exposed wild crustaceans is more likely compared to most other hazards. The estimations for the other hazards were considered appropriate.	
12	*"Use of Imported prawns as feed for crustacean broodstock"* as a major exposure pathway should be removed as the practice of feeding prawns to broodstock is no longer practiced in Australia.	**Noted and agreed.** The department considers that the use of imported prawns as feed in farms or hatcheries is an unlikely exposure pathway. However, if it were to occur it would be a direct and potentially significant exposure pathway and it is therefore retained as a major exposure pathway. Based on the strong views of some stakeholders that this practice does not occur on farms or in hatcheries in Australia, the department has reduced the exposure ratings for the farmed exposure group. The exposure likelihoods for the hatchery exposure groups have not been reduced. This is because the hatchery exposure group also contains animals kept in public aquaria and research institutes.	4.3 Exposure assessment Appendix D 2) Use of imported prawns as feed for crustacean broodstock and crustaceans in research facilities and public aquaria All risk assessment chapters
13	The PLES and the overall impact of establishment and spread for wild crustaceans in the draft report have been underestimated. This includes impacts to prawn aquaculture, broodstock supply, fisheries, prawn bait industry domestic and international trade.	**Noted**. The likelihood that a hazard will establish, and spread is affected by how easily the hazard can be transmitted from an index case to other susceptible species. In the wild, consumption of diseased prawns by non-susceptible animals may reduce the likelihood of establishment and spread. Factors such as an environment conducive to increased protection from non-susceptible predators would increase the likelihood of establishment and spread in a wild population. The overall impact of each hazard is considered for the entire Australian community, rather than on the directly affected parties. For most hazards there is little evidence to suggest there have been impacts on wild populations. However, it was assumed that wild prawns (including native species) in Australia would be at least as susceptible to infection as prawns of the same species, reported as susceptible under similar conditions in other countries.	8 Decapod iridescent virus 1 risk review 9 *Enterocytozoon hepatopenaei* risk review 14 White spot syndrome virus risk review

Issue number	Issue	Department response	Final report reference
		The risk assessment for all hazards, including the consequence assessments have been reviewed. The PLES of DIV1 in wild crustaceans was reconsidered and has increased due to new information on the host range and transmissibility of DIV1. The PLES of EHP in wild crustaceans has increased due to new information indicating a broader host range and ability to remain infectious in infected prawns and the environment for extended periods. The PLES of WSSV in wild crustaceans was reconsidered and increased due to its broad host range and transmissible through water, the establishment and spread of WSSV in the directly exposed wild crustaceans is more likely compared to most other hazards. The estimations for the other hazards were considered appropriate.	
14	The consequence of the loss of disease-free status because of the inability to eradicate an aquatic animal disease in the aquatic environment has been underestimated.	**Noted.** The draft report acknowledged that for all hazards the economic effects of new or modified eradication, control, monitoring or surveillance and compensation strategies or programs to regain disease-free status would result in minor impacts at the national level. It is also acknowledged that loss of disease-free status for the hazards may impact on international trade. For most hazards there is little evidence to suggest that there have been impacts on wild populations. The risk assessment for all hazards, including the adverse impacts, have been reviewed. The estimations were considered appropriate.	No changes to the final report.
15	The infrastructure required to prevent hazards entering and leaving a farm via water is unlikely to be present in farms to prevent entry and exit of many of the exotic hazards.	**Noted.** The report acknowledges that not all farms have the same standards of biosecurity infrastructure, and the risk assessments already take this into account. Therefore, no changes to the PLES ratings have been made because of this factor. The wording around this issue has been strengthened.	4.3.3 Key considerations of the exposure assessment
16	Prawn farm effluents in Australia are not always treated through settlement, dilution or screening before their release into natural waters.	**Noted and agreed.** The department acknowledges this new information provided by stakeholders and has taken it into consideration. A conservative approach was already taken with consideration of farm biosecurity	Appendix D Infectious dose

Issue number	Issue	Department response	Final report reference
		practices such as this and therefore it was not considered necessary to adjust the partial likelihood of establishment and spread.	
17	For hazards included in the review, both, the PLE and partial annual likelihood of entry and exposure (PALEE) should be estimated to be High in cases where PLE is estimated to be High for the exposure group "wild crustaceans", susceptible species are present in wild populations; and known persistent major exposure pathways exist.	**Noted.** The PLE of each exposure group to a hazard in imported prawns was estimated based on available relevant scientific evidence. PALEE for each exposure group is determined using the *matrix for combining descriptive likelihoods* by combining the likelihood of entry (LR) and the PLE. The PLE of wild crustaceans to DIV1, WSSV and YHV1 was considered **High**. For other hazards for which the host range is narrower than that of DIV1, WSSV and YHV1, the likelihood of exposure of wild crustaceans was considered to be lower. The probability of wild crustaceans encountering imported prawns (or associated wastes) depends on several factors such as the volume of product released into the natural environment, the dispersal and dilution of that material and the proportion of material that might be consumed by other non-susceptible species in the vicinity. The PLE of wild crustaceans to DIV1 has increased from **Moderate** to **High**. This is due to new information indicating a broader host range under natural conditions. The estimations for the other hazards were considered appropriate.	8.3.2 Exposure assessment
-	**Options for biosecurity management of imported prawns**	-	-
-	Sourcing from free populations/ Sourcing from wild stocks	-	-
18	Biosecurity risk cannot be managed through sourcing from free populations or wild fisheries (if considered equivalent).	**Noted.** To be satisfied that a country, compartment or zone is free of a given disease, the department must have formally recognised the competent authority (CA) of that country and be satisfied that the competent authority has the capacity for disease control, monitoring and surveillance as appropriate for the disease. Determination of hazard freedom would need to be to a standard consistent with that recommended by the WOAH, or equivalent. Assessments would be done on a case-by-case basis and if approval is given may require verification activities at the border to ensure import conditions are being met.	5.1.2 Sourcing from wild stocks

Issue number	Issue	Department response	Final report reference
		The department considers that some hazards, including WSSV, are present at a prevalence and load in wild populations that is of biosecurity concern to the department. Therefore, consideration of alternative biosecurity measures for prawns sourced from wild stocks will require a case-by-case assessment in-line with the requirements for *Sourcing from free populations*. The section about sourcing from wild stocks has been updated to strengthen that this measure would only be considered following an assessment of disease freedom.	
-	Cooking	-	-
19	Cooked prawns are being used as bait and berley, and it is likely that the proportion of fishers using cooked prawns will have increased on the assumption that cooked prawns do not pose a biosecurity risk.	**Noted**. Whether cooked prawns are used as bait was a question included in the *2019–20 National social and economic recreational fishing survey–prawn use by recreational fishers for bait and berley.* The survey found that nationally, 42% of survey respondents who used some type of bait, used prawns as bait in the 12 months prior to the survey. Of those, 20.3% indicated that they had purchased the prawns from seafood retailers. Of the respondents who purchased prawns as bait from a seafood retailer, 41% reported using cooked but not shelled prawns and 32% reported using cooked and shelled prawns as bait. The department has considered this data in the final report. The survey outcomes and the way in which they affected previous conclusions presented in the draft report had been also considered. Although cooked prawns were not the preferred form of prawns used as bait or berley by recreational fishers in Australia, the usage of cooked prawns was greater than that assumed in the draft report. The usage of cooked prawns for bait is similar to that for uncooked, shelled prawns. Consequently, it is now assumed that cooking reduces the likelihood of exposure of wild crustaceans to an extent similar to head and shell removal. Changes in the exposure likelihoods for cooked prawns have been done accordingly.	1.6 Use of prawns as bait and berley by recreational fishers Appendix D Use of imported prawns as bait or berley for recreational fishing All risk assessment chapters - X.4.2 Cooking
20	Minimum times and temperatures for cooking prawns should be specified, as the existing guidance for cooking (>70°C core temperature for >11 seconds) has insufficient detail and can be subject to fraud.	**Noted.** Cooking as a biosecurity measure has been reconsidered for all hazards. Changes have been made to the cooking requirements.	5.1.3 Cooking All risk assessment chapters - X.4.2 Cooking 16.3.1 Cooked prawns

Issue number	Issue	Department response	Final report reference
		To ensure cooked prawns achieve Australia's ALOP for all hazards, the department considers prawns to be cooked when they appear fully cooked and have achieved a core temperature of at least 65°. Cooking, with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C, would generally be expected to reduce both entry and exposure likelihoods for the hazards. The extent to which this would occur is dependent upon the specific hazard and has been assessed for each hazard based upon the available data regarding hazard infectivity following heat treatment. Minimum cooking time is not specified for cooked prawns. However, the CA must be able to certify that the prawns appear fully cooked and that they have achieved a core temperature of at least 65°C. Cooking as a risk management section in the final report has been revised to include information about the monitoring of cooked prawns.	
21	Border inspection of cooked prawns and adequate levels of 'seals intact' inspections are needed.	**Noted.** Whilst cooked prawn consignments do not undergo a 100% seals-intact inspection on-arrival, cooked prawn consignments can undergo random seals-intact fully supervised inspections at a rate determined by the department. If the risk for cooked prawns' changes, the inspection rates will be reassessed.	-
-	Value-added product	**-**	-
22	The overall restricted risk of imported uncooked prawns which have been processed into a value-added product (VAP), such as breaded, battered and crumbed (BBC) or dumpling and dim sum-type products, is low and conditions like an import permit should not be mandatory for each consignment	**Noted.** Value-added products are products in which the uncooked prawns have had the head and shell removed and have been further processed into a BBC product or a dumpling and dim sum-type product. Specific import conditions apply to each product type for them to meet the definition of a 'highly processed prawn' and an import permit is required. By law, all uncooked prawns or products containing uncooked prawns, require an import permit which specifies the import conditions required to mitigate their biosecurity risk. This is because uncooked prawns or products containing uncooked prawns are a conditionally non-prohibited good.	No changes to the final report.

Issue number	Issue	Department response	Final report reference
23	Evidence showing that VAP are unlikely to be used as bait or berley compared to unprocessed prawns needs to be provided.	**Noted.** The overall restricted risk of imported uncooked prawns which have been processed into a VAP, was determined to achieve Australia's appropriate level of protection (ALOP) for all pathogenic agents of biosecurity concern in the review. This is mainly because the likelihood of exposure to VAP would be significantly reduced as VAP are unlikely to be used as bait or berley compared to unprocessed (whole or head and shell off) prawns and the general higher cost of VAP. This assumption is supported by the data from the *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley report* (Moore et al. 2021). There was a total of 23,747 participants in the online component of the survey. • From the 23,747 participants, 17,596 responded to questions regarding their fishing participation. • A subset of 11,849 participants indicated they fished within the last 12 months. • A further subset of 5,514 recreational fishers indicated they had used any type of bait in the previous 12 months. • Those who used prawns or shrimp (3,396) were asked if they had purchased the prawns from a seafood retailer or a bait supplier. • Those recreational fishers who reported using prawns purchased from a seafood retailer (822) were asked about the prawn (product) type, source (Australian origin or imported) and reasons for purchasing prawns from a seafood retailer rather than a bait supplier. It was found that nationally, 42% of survey respondents who used some type of bait, used prawns as bait in the 12 months prior to the survey. Of those, 20.3% indicated that they had purchased the prawns from seafood retailers. The survey found that of the respondents who purchased prawns as bait from a seafood retailer in the 12 months prior to the survey: • 82% reported using whole, uncooked prawns (58% reporting using <1 kg: and 24% reporting using >1 kg).	1.6 Use of prawns as bait and berley by recreational fishers 4.3.3 Key considerations of the exposure assessment Appendix D Use of imported prawns as bait or berley for recreational fishing

Issue number	Issue	Department response	Final report reference
		• 40% reported using uncooked but shelled prawns (34% reporting using < 1 kg; 6% reporting using > 1 kg). • 41% reported using cooked but not shelled prawns (27% reporting using < 1 kg; 14% reporting using > 1 kg). • 32% reported using cooked and shelled prawns as bait (22% reporting using < 1 kg; 10% reporting using > 1 kg). • 24% reported using processed prawns (for example, skewers, marinated, breaded or battered, part of a dumpling, spring roll or other product, or butterflied) (18% reporting using <1 kg; and 6% reporting using > 1 kg) (Moore et al. 2021). The results of the 2019–20 *National recreational fishing survey–prawn use by recreational fishers for bait and berley* report are not significantly different from the assumptions for VAP presented in the draft report and therefore exposure likelihoods have not changed in this final report.	
24	Further assurance that recategorization of prawns as cooked or par-cooked BBC prawns will not result in release of a hazard need to be provided.	**Noted.** BBC and cooked prawns are less likely to be used as bait and berley compared to uncooked prawns which reduces the likelihood of exposure of the hazards for these products. This assumption is supported by the data from the *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* report (Moore et al. 2021). There was a total of 23,747 participants in the online component of the survey. • From the 23,747 participants, 17,596 responded to questions regarding their fishing participation. • A subset of 11,849 participants indicated they fished within the last 12 months. • A further subset of 5,514 recreational fishers indicated they had used any type of bait in the previous 12 months. • Those who used prawns or shrimp (3,396) were asked if they had purchased the prawns from a seafood retailer or a bait supplier. • Those recreational fishers who reported using prawns purchased from a seafood retailer (822) were asked about the prawn	1.6 Use of prawns as bait and berley by recreational fishers Appendix D Use of imported prawns as bait or berley for recreational fishing

Issue number	Issue	Department response	Final report reference
		(product) type, source (Australian origin or imported) and reasons for purchasing prawns from a seafood retailer rather than a bait supplier. It was found that nationally, 42% of survey respondents who used some type of bait, used prawns as bait in the 12 months prior to the survey. Of those, 20.3% indicated that they had purchased the prawns from seafood retailers. The survey found that of the respondents who purchased prawns as bait from a seafood retailer in the 12 months prior to the survey: • 82% reported using whole, uncooked prawns (58% reporting using <1 kg: and 24% reporting using >1 kg). • 40% reported using uncooked but shelled prawns (34% reporting using < 1 kg; 6% reporting using > 1 kg). • 41% reported using cooked but not shelled prawns (27% reporting using < 1 kg; 14% reporting using > 1 kg). • 32% reported using cooked and shelled prawns as bait (22% reporting using < 1 kg; 10% reporting using > 1 kg). • 24% reported using processed prawns (for example, skewers, marinated, breaded or battered, part of a dumpling, spring roll or other product, or butterflied) (18% reporting using <1 kg; and 6% reporting using > 1 kg) (Moore et al. 2021). The results of the *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* report are not significantly different from the assumptions presented in the draft report and therefore exposure likelihoods for VAP have not changed in this final report. Skewered, butterflied and marinated prawns are considered as uncooked prawns by the department, despite there being a level of processing. Therefore, the usage of breaded, battered and crumbed and dumpling-type products as bait is likely less than what is reported in these usage patterns.	
25	The risk of exposure of prawns processed into a VAP via the berley pathway is not **negligible** for some hazards.	**Noted.** The PLE of wild crustaceans to VAP was estimated as **Extremely Low** for all hazards, not **Negligible**.	1.6 Use of prawns as bait and berley by recreational fishers Value-added products section of each risk assessment chapter.

Issue number	Issue	Department response	Final report reference
		These likelihood estimations consider the data obtained from the *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley*. Where 24% of survey respondents who used prawns purchased from a seafood retailer, reported using processed prawns (for example, skewers, marinated, breaded or battered, part of a dumpling, spring roll or other product, or butterflied) (18% reporting using <1 kg; and 6% reporting using > 1 kg) (Moore et al. 2021). It is noted that in the context of the definition of a processed prawn in the survey, marinated, skewered prawns are included, these product types are not considered a VAP for the purposes of biosecurity risk management and therefore it is assumed that the amount of VAP used for bait and berley is also less than identified in the survey. The results of the *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* report are not significantly different from the assumptions for VAP presented in the draft report, and therefore exposure likelihoods for VAP have not changed in this final report.	
-	Head and shell removal	-	-
26	Head and shell removal of uncooked prawns does not manage the risk for covert mortality nodavirus (CMNV), DIV1, infectious myonecrosis virus (IMNV), Laem-Singh virus (LSNV), Taura syndrome virus (TSV) and (Vp AHPND) because the hazard titre is not reduced.	**Noted.** All the risk assessments have been reviewed and updated when needed. The removal of the head and shell from uncooked prawns is expected to significantly reduce the viral load of DIV1, however, sufficient virus to cause infection may still be present. DIV1 was not detected in deveined, de-headed and de-shelled prawns during monitoring programs undertaken during 2020–22. Because DIV1 was not detected in these programs, the entry likelihood for DIV1 in these products is reduced based on the known approach rates of DIV1. Ongoing monitoring of imported batches for DIV1 is continuing to ensure that any changes to the approach rates of DIV1 is known and additional biosecurity measures can be applied, as necessary. The removal of the head and shell from uncooked prawns is expected to reduce the likelihood of entry for LSNV and Vp AHPND which are present primarily in the head and shell.	7 Covert mortality nodavirus risk review 8 Decapod iridescent virus 1 risk review

Issue number	Issue	Department response	Final report reference
		The prawn review considered that all the other hazards in untested, de-headed and de-shelled prawns would be present in sufficient doses to cause infection if a susceptible species is exposed to them. However, due to the reduction in likelihood of use as bait by recreational fishers, and therefore a reduction in the exposure likelihoods, the risk for IMNV and TSV achieves ALOP. In the case of CMNV, the removal of the head and shell is not expected to reduce the load of CMNV in deveined, de-headed and de-shelled prawns. However, as CMNV was also included in the monitoring programs undertaken during 2020–22 and it was not detected, the entry likelihood is reduced based on the known approach rates of CMNV. Although farmed and hatchery crustaceans were considered unlikely to be exposed to whole, uncooked prawns as feed for maturation purposes, head and shell removal is expected to further reduce the likelihood of this occurring because it removes the nutritional benefit associated with this practice. There may be some minor use of head and shell off prawns as feed for aquatic animals held in research or public aquaria. The review assumed that the removal of the head and shell will not significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. The results of the 2019–20 *National recreational fishing survey–prawn use by recreational fishers for bait and berley* showed that prawns which were uncooked and had been shelled were less likely to be used as bait or berley compared to whole uncooked prawns. It was found that nationally, 42% of survey respondents who used some type of bait, used prawns as bait in the 12 months prior to the survey. Of those, 20.3% indicated that they had purchased the prawns from seafood retailers. Of the respondents who purchased prawns as bait from a seafood retailer in the 12 months prior to the survey, 82% reported using whole, uncooked prawns (58% reporting using <1 kg: and 24% reporting using >1 kg) compared to 40% who reported using uncooked but shelled prawns (34% reporting using < 1 kg; 6% reporting using > 1 kg). Because the results of the survey were different from the assumptions presented in the draft report, the exposure likelihoods for wild prawns	

Issue number	Issue	Department response	Final report reference
		to uncooked prawns which have had the head and shell removed have been changed in this final report.	
27	Removing the head and shell of the prawn make it more attractive to use as bait/berley.	**Noted.** This statement is not supported by the outcomes *of 2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* which showed that prawns which were uncooked and had been shelled were less likely to be used as bait or berley compared to whole uncooked prawns. It was found that nationally, 42% of survey respondents who used some type of bait, used prawns as bait in the 12 months prior to the survey. Of those, 20.3% indicated that they had purchased the prawns from seafood retailers. Of the respondents who purchased prawns as bait from a seafood retailer in the 12 months prior to the survey, 82% reported using whole, uncooked prawns (58% reporting using <1 kg: and 24% reporting using >1 kg) compared to 40% who reported using uncooked but shelled prawns (34% reporting using < 1 kg; 6% reporting using > 1 kg). The final report has been updated to include the outcomes and results from the *National social and economic recreational fishing survey*.	1.6 Use of prawns as bait and berley by recreational fishers 4.3.3 Key considerations of the exposure assessment Appendix D Use of imported prawns as bait or berley for recreational fishing
-	Deveining	-	-
28	Deveining of uncooked prawns only satisfactorily reduces disease risk for some pathogens and is not an adequate management measure in addition to removing the head and shell.	**Noted.** The risk review for *Enterocytozoon hepatopenaei* (EHP) has be reconsidered on the basis of new information available about the impact of freezing on EHP infectivity. The University of Arizona was commissioned to conduct independent trials investigating the effect of commercial freezing temperatures on the infectivity of EHP. The results showed that freezing at –18°C for 24 hours does not completely inactivate EHP, however the results also showed that there is a reduction in pathogen ability to cause infection as freezing time increases. Risk review was conducted assuming only a 24 hour freezing time for imported prawns, as a result the unrestricted risk of imported prawns (that is frozen and whole) did not achieve Australia's ALOP. Deveining is one of a suite of biosecurity measures used in combination with freezing and head and shell removal. Deveining (removal of the digestive tract to at least the last shell segment) only manages the biosecurity risks associated with EHP. By	9 *Enterocytozoon hepatopenaei* risk review

Issue number	Issue	Department response	Final report reference
		removing the head, shell and digestive track of uncooked prawns, it is expected that the likelihood of entry of EHP will be reduced due to the reduction in the number of EHP spores present in the prawns, when combined with the reduction in infectivity due to freezing, Australia's ALOP is achieved. The restricted risk assessment for EHP has been reviewed and updated according to this new information. Head and shell removal plus deveining is necessary to reduce the overall risk of EHP in frozen prawns to meet Australia's ALOP.	
29	Deveining should not be included as a biosecurity measure to control EHP spores for frozen prawn and prawn products because NACA has reported that EHP spores will be inactivated at -20°C for 2 hours.	**Noted.** Given the information provided at NACA webinars in 2021, the department commissioned independent research by the University of Arizona's Aquaculture Pathology Laboratory to undertake bioassays to confirm that freezing at commercial freezing temperatures inactivates EHP in commodity prawns. University of Arizona conducted trials investigating the effect of freezing the hepatopancreas of EHP-affected prawns at – 18°C for 24 hours, 7 days and 14 days on the ability of the EHP to cause infection in *P. vannamei* through oral challenge. The results showed that freezing at –18°C for 24 hours does not completely inactivate EHP, however the results also showed that there is a reduction in pathogen ability to cause infection as freezing time increases. The restricted risk assessment for EHP has been reviewed and updated according to this new information. Although freezing played a significant role in managing biosecurity risks of EHP in imported prawns by reducing the likelihood of entry (refer section 9.3.1 Entry assessment), freezing on its own, does not completely remove the risk when a 24 hour freezing period is assumed, and Australia's ALOP was not achieved. Head and shell removal plus deveining is required to reduce the overall risk of EHP to meet Australia's ALOP.	9.2.1 Agent properties 9.4.2 Head and shell removal plus deveining
-	Pre-export and on-arrival batch testing	-	-
30	The review needs to explain how failures in the testing regime will be addressed.	**Noted and agreed.** Since the white spot disease (WSD) outbreak in South-East Queensland in 2016–17, improvements have been made to the on-arrival testing system, the laboratory testing scheme and new inspection procedures	17 Testing of imported prawns

Issue number	Issue	Department response	Final report reference
		are in place, as well as the addition of testing prior to export of prawns to Australia. Additionally, the current testing and sampling regimes are being further considered by experts in the subject. A new chapter has been included in the final report with detailed information regarding the testing program for imported prawns.	
31	Pre-export batch testing is not a reliable biosecurity measure and should not be considered**.**	**Noted.** The report has only considered pre-export batch testing as a biosecurity measure in combination with head and shell removal (in the circumstance that deveining, and head and shell removal does not achieve Australia's ALOP). Through a scientific risk assessment, it was determined that post-processing batch testing of prawns for WSSV and YHV1 prior to export would reduce the likelihood of entry. But under this scenario it is assumed that the testing is not conducted under a department approved testing or sampling system. This reduction of the likelihood of entry is not therefore sufficient to reduce the overall restricted risk of WSSV and YHV1 to achieve Australia's ALOP. Therefore, additional on-arrival batch testing was considered necessary for WSSV and YHV1. A new chapter has been included in the final report with detailed information regarding the testing program for imported prawns, including information about pre-export batch testing.	17 Testing of imported prawns
32	The testing program needs to be expanded to include emerging pathogenic agents.	**Noted.** The department has undertaken a program to monitor for emerging diseases. The department engaged the Australian Centre for Disease Preparedness (ACDP) to undertake testing of batches of imported uncooked prawns for DIV1, EHP and CMNV. These hazards were selected as the department considered them emerging pathogens and that there was a lack of information about their presence in imported prawns and their geographical distribution. The program provided information that was used when considering the risk assessments for DIV1, EHP and CMNV. A similar program will also be used for the foreseeable future to monitor for the presence of other pathogenic agents or to continue monitoring these emerging diseases in imported uncooked prawns.	17.4 Monitoring imported prawns for emerging diseases

Issue number	Issue	Department response	Final report reference
		The department based its risk assessments on the available science. It was not determined that batch testing for additional hazards was required to achieve Australia's ALOP. If the biosecurity risks change and a risk assessment determines it is necessary, the department could and would implement a testing regime like what is in place for WSSV and YHV1.	
33	Detail is needed around the way batch sampling and PCR testing manage risk and how they can be verified. There are concerns about the suitability of using a design prevalence 5%, as well as concerns about the testing regime not providing adequate confidence of freedom from WSSV and YHV1 and not being consistent with WOAH recommendations.	**Noted and agreed.** As WSSV and YHV1 are highly transmissible, a prevalence of 5% was selected for the on-arrival sampling procedures for imported uncooked prawns, which is consistent with the criteria in the WOAH Code for disease freedom surveillance. Similarly, the sampling program is designed to provide 95% confidence of pathogen detection, which is also consistent with the recommendation in the WOAH Code that the level of confidence in the surveillance system should be greater than or equal to 95%. The department engaged with statistical experts within the Australian Bureau of Agricultural and Resource Economics and Sciences and an independent statistical expert at the Australian National University to review the imported prawn sampling design and make any necessary adjustments to the sampling program design to maintain the desired level of confidence. A new chapter has been included in the final report with detailed information regarding the testing program for imported prawns, including sampling, testing and verification activities.	17 Testing of imported prawns
34	Testing of imported prawns should be undertaken by the department to determine the true prevalence of identified hazards.	**Noted.** The department has conducted a generic risk assessment which assumes the hazards are present in all countries and present in the prawns at the time they are imported. Countries may request an assessment from Australia to import prawns that are recognised as free of pathogenic agents of biosecurity concern. This could be for sourcing from free populations such as country, zone or compartment. The department has undertaken a program to monitor for emerging diseases in imported uncooked prawns and a similar program will also be used for the foreseeable future.	17.4 Testing of imported uncooked prawns for emerging diseases

Issue number	Issue	Department response	Final report reference
		The department also undertakes testing of imported prawns from Australian retail outlets to verify that Australia's current import conditions for prawns are being met. A new chapter has been included in the final report with detailed information regarding the testing program for imported prawns, including the monitoring program and the retail testing for WSSV.	
35	On-arrival testing is trade restrictive and it should not be reconsidered as a biosecurity measure as the competent authority in the exporting country already certifies that the uncooked prawns are found post-processing to be free of WSSV and YHV1 on the health certificate.	**Noted.** Through a scientific risk assessment, the department determined that the overall restricted risk of WSSV and YHV1 in imported prawns with the biosecurity measure, head and shell removal in combination with pre-export testing applied, did not achieve Australia's ALOP. The inclusion of on-arrival batch testing is needed for the overall restricted risk of both hazards to achieve Australia's ALOP. If the department were to assess an exporting country's pre-export testing systems and it was satisfied that it met the required standards, then the level of testing applied on-arrival could be reduced through a compliance-based interventions scheme (CBIS).	No changes to the final report.
-	Labelling	-	-
36	Labelling of imported prawns "for human consumption only" and "not to be used as bait or feed for aquatic animals" is not an adequate biosecurity measure.	**Noted.** Labelling is not used as a standalone biosecurity measure. The department's view is that labelling should be included as an import condition because any reduction in unintended end-use or deliberate diversion, for example as bait, is beneficial in reducing risk. Labelling also forms part of an on-going education campaign and is one of a suite of biosecurity measures used in combination.	No changes to the final report.
37	Labelling alone is sufficient to manage biosecurity risk.	**Noted.** The department does not consider that labelling alone manages biosecurity risk to a level that achieves Australia's ALOP. This is supported by the outcomes of the *National social and economic recreational fishing survey*. The survey found that nationally, 20.3% of respondents indicated that they had purchased bait from seafood retailers in the previous 12 months. The survey also found that 24.1% of respondents who purchased bait from seafood retailers in the previous 12 months sometimes used imported prawns and 9.4% often	No changes to the final report.

Issue number	Issue	Department response	Final report reference
		used imported prawns, a further 19.7% did not know if they had used imported prawns. The department's view is that labelling should be included as an import condition because any reduction in unintended end-use or deliberate diversion, for example as bait, is beneficial in reducing risk. Labelling also forms part of an on-going education campaign and is one of a suite of biosecurity measures used in combination.	
-	Uncooked wild-caught prawns of Australian origin processed overseas in approved premises	-	-
38	Australian farmed prawns exported overseas for processing and re-imported should have import requirements similar to current import conditions for wild Australian prawns processed overseas in a departmental approved facility.	**Noted.** Current import conditions for '*Uncooked Australian prawns processed overseas in a departmental approved facility*' support a specific agreement for Australian origin wild-caught prawns exported to Thailand to be processed in the Thai Union Frozen Products Public Company Ltd facility and re-imported into Australia. At the time of writing, this is the only prawn processing facility approved by both the department and Thailand's CA for this pathway. Australian origin prawns imported through this pathway are subject to a risk-based reduction for on-arrival testing requirements. Australian origin farmed prawns processed overseas in a departmental approved facility for re-import into Australia would be assessed in the same way as Australian origin wild-caught prawns. In each case a competent authority must demonstrate that a secure supply chain and processing arrangement is in place to prevent contamination or substitution with non-Australian origin prawns. The department will consider submissions from a competent authority supporting equivalence measures for the secure processing of Australian farmed or wild-caught prawns, on a case-by-case basis.	No changes to the final report.
-	Irradiation	-	-
39	Irradiation of uncooked prawns should be considered as a reasonable means of risk minimization and as a middle ground to cooking.	**Noted.** Food Standards Australia and New Zealand (FSANZ) approves the use of irradiation of food intended for human consumption. Irradiation of prawns for human consumption is not currently approved. If FSANZ were to approve irradiation of prawns and prawn products intended	5.2.5 Irradiation

Issue number	Issue	Department response	Final report reference
		for human consumption, the department would review whether irradiation as a biosecurity measure achieved Australia's ALOP. A section regarding the consideration of irradiation as a biosecurity measure has been included in the final report.	
-	**Hazard risk reviews**	-	-
-	"*Candidatus* Hepatobacter penaei"	-	-
40	The PLE, PLES and impacts for wild crustaceans were underestimated for *"Candidatus* Hepatobacter penaei" (*"Ca.* H. penaei"). The risk assessment needs to be reviewed.	**Noted.** The department reviewed the risk assessment for "*Ca.* H. penaei". including the estimations for PLE, PLES and impacts of an outbreak of "*Ca.* H. penaei". The estimation for PLE was considered appropriate for frozen prawns. This is primarily due to the narrow host range of *"Ca.* H. penaei" and the impact of freezing on *"Ca.* H. penaei". However, the PLE of farmed prawns to *"Ca.* H. penaei" in imported chilled prawns has been reduced from **Low** to **Very low.** This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds. The overall impact and PLES estimated for *"Ca.* H. penaei" was determined to be appropriate considering the available information about its impacts overseas and the narrow host range present in Australia.	6 *"Candidatus* Hepatobacter penaei" risk review
-	Covert mortality nodavirus	-	-
41	A wider range of finfish should be considered susceptible to CMNV for consistency with other assessments carried out in the department such as the *Draft update of non-salmonid marine finfish risk species lists for human consumption and baitfish*.	**Noted and agreed.** The department has included reports in the risk assessment about CMNV infecting finfish species, including *Carassius auratus* and *Danio rerio*, which are present in Australia. It is acknowledged that there are species related to CMNV susceptible finfish at the genus level or higher which are native to Australia. However, the review follows the criteria in Article 1.5.9 of the WOAH Code for *Listing susceptible species at a taxonomic ranking of Genus or higher*. The CMNV susceptible finfish species do not meet this criterion. Should information become available that suggests finfish species	4.3.1 Identification of exposure groups 7 Covert mortality nodavirus risk review

Issue number	Issue	Department response	Final report reference
		native to Australia are susceptible to CMNV, the department will reconsider the risk assessment for CMNV.	
42	Change the exposure group '*Wild crustaceans*' to include fish species that are susceptible to CMNV.	**Noted and agreed**. The definition of the exposure groups in the CMNV risk assessment has been changed to take into account reports of CMNV infecting the finfish species *C. auratus* and *D. rerio* which are present in Australia.	7 Covert mortality nodavirus risk review
43	Review the assessment for the PLE for CMNV.	**Noted and agreed**. Since the release of the draft report, there is new information that CMNV infects two finfish species reported in Australia, *C. auratus* and *D. rerio*. Taking this new information into account the risk review has been reviewed. The PLE of farmed susceptible species to CMNV in imported prawns has been reduced from **Low** to **Very low.** This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds. *C. auratus* and *D. rerio* are present in ornamental finfish facilities and research institutions but it was considered unlikely they would be fed imported prawns. *C. auratus* are found in the wild in Australia and may be directly exposed to imported prawns used as bait and berley but it was not considered to appreciably change the risk rating for wild susceptible species compared to the rating in the draft report.	7 Covert mortality nodavirus risk review
44	For the CMNV risk assessment, provide evidence to justify the selection of the less conservative PLES for wild crustaceans of Very Low. If evidence is not available and there is significant uncertainty, a more conservative likelihood should be used.	**Noted**. The risk assessment for CMNV has been reconsidered and the estimations for PLES are appropriate. The range of species susceptible to CMNV which are present in Australia is narrower than for other hazards. The new information that *C. auratus* and *D. rerio* are susceptible to CMNV was considered, but as they are freshwater ornamental finfish that are unlikely to be present near prawn farms it was determined they would not appreciably contribute to the PLES.	7 Covert mortality nodavirus risk review
45	The overall risk rating for CMNV should be higher and batch testing should be required to manage the risk.	**Noted.** The risk assessment for CMNV has been reviewed.	7 Covert mortality nodavirus risk review 17 Testing of imported prawns

Issue number	Issue	Department response	Final report reference
		New information about CMNV infecting finfish species present in Australia has been incorporated into the risk assessment. *C. auratus* and *D. rerio* are freshwater fishes that are unlikely to be present near prawn farms. Therefore, it was determined they would not appreciably contribute to the PLE or PLES for wild susceptible species. The risk rating for PLE of farmed susceptible species has been reduced from **Low** to **Very Low** considering new information that farmed susceptible species are unlikely to be intentionally fed imported prawns. The overall impact estimated for CMNV was determined to be appropriate taking into account the available information about its impacts overseas. The overall annual risk associated with CMNV was determined to be **Low**. This is consistent with the annual risk for CMNV in the draft report. As the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard. Head and shell removal was considered to reduce the overall annual risk of CMNV to **Negligible** which achieves Australia's ALOP. Therefore, batch testing was not required. The department also undertook monitoring of imported prawns for CMNV. Of the 90 batches tested, none were positive for CMNV. The department intends to maintain a monitoring program for imported prawns for the foreseeable future. Should information become available that suggests the biosecurity risk of CMNV has changed, the department will reconsider the risk assessment for CMNV.	
46	Review the assessment with biosecurity measure cooking applied.	**Noted**. The restricted risk assessment has been reconsidered and updated based on revision of import conditions for cooked prawns, in the context of the outcomes of the bait and berley survey and the cooking studies undertaken by the University of Arizona. As there is no new information about heat inactivation of CMNV it was still considered that cooking may reduce, but not completely inactivate CMNV in imported prawn tissues. Some infectious virus may remain.	7 Covert mortality nodavirus risk review 7.4.2 Cooking

Issue number	Issue	Department response	Final report reference
		Cooking to attain a core temperature of at least 65°C is expected to reduce the likelihood of entry, but not completely remove it.	
-	Decapod iridescent virus 1	-	-
47	Review the assessment for the PLE for DIV1.	**Noted**. The PLE of farmed crustaceans to DIV1 reduced from **Low** to **Very Low**. This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds. The PLE for the hatchery exposure group did not change because it was assumed that a very small, yet significant volume of whole uncooked prawns could be used as feed for crustaceans in public aquaria and research facilities. The PLE of wild crustaceans to DIV1 increased from **Moderate** to **High.** This is because *Penaeus monodon* and *Ch*erax *quadricarinatus* are present in the wild in Australia and new information suggests that DIV1 can cause infection and mortalities in these species.	8 Decapod iridescent virus 1 risk review
48	For the DIV1 risk assessment, provide evidence to justify selection of the less conservative PLES for wild crustaceans Very Low. If evidence is not available and there is significant uncertainty, the more conservative likelihood should be used	**Noted**. The PLES of DIV1 in farmed crustaceans increased from **Moderate** to **High**. This is because of the new information on the susceptibility of *P. monodon* to DIV1 infection, the infectious dose of DIV1, the tissue distribution of DIV1 and the existing information that DIV1 is transmitted horizontally through ingestion of infected tissues and likely via water. The PLES of DIV1 in wild crustaceans increased from **Very Low** to **Low**. This is because *P. monodon, C. quadricarinatus* and the crab species *Portunus trituberculatus* are present in the wild in Australia and new information suggests that DIV1 can cause infection and mortalities in these species.	8 Decapod iridescent virus 1 risk review
49	The overall risk rating for DIV1 should be higher and batch testing should be required to manage the risk.	**Noted.** The risk assessment for DIV1 has been reviewed. Since the release of the draft report, there is new information about DIV1 infecting and causing mortalities in *P. monodon* and C*. quadricarinatus*. Both are important farmed and wild-caught species in Australia.	8 Decapod iridescent virus 1 risk review

Issue number	Issue	Department response	Final report reference
		The PLE of farmed crustaceans to DIV1 has been reduced from **Low** to **Very Low** considering new information that farmed crustaceans are considered unlikely to be intentionally fed imported prawns. The PLE of wild crustaceans to DIV1 has increased from **Moderate** to **High** to account for the increased exposure risk to *P. monodon, C. quadricarinatus* and *P. tritubercula*tus present in the wild in Australia. The PLES of DIV1 in farmed crustaceans increased from **Moderate** to **High** due to the susceptibility of *P. monodon* to DIV1 and because farming of *P. monodon* accounts for the majority of prawn farming production in Australia. The PLES of DIV1 in wild crustaceans increased from **Very low** to **Low** as DIV1 can cause infection and mortality in *P. monodon, C. quadricarinatus* and *P. tritubercula*tus. With these changes, the overall annual risk associated with DIV1 was determined to be **Low**. This is consistent with the overall risk for DIV1 in the draft report. As the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for this hazard. In the restricted risk assessment for DIV1, the entry likelihood for DIV1 in deveined, de-headed and de-shelled prawns was reduced from **High** to **Moderate**. This is based on the known approach rates of DIV1 in deveined, de-headed and de-shelled prawns. DIV1 was not detected during monitoring programs undertaken during 2020–22 in these products. These programs included batches from countries where DIV1 is endemic. Ongoing monitoring of imported batches for DIV1 is continuing to ensure that any changes to the approach rates of DIV1 is known and additional biosecurity measures can be applied, as necessary.	
-	*Enterocytozoon hepatopenaei*	-	-
50	The PLE, PLES and impacts for wild crustaceans were underestimated for EHP. The risk assessment needs to be reviewed.	**Noted**. The PLE of farmed crustaceans to EHP reduced from **Low** to **Very Low**. This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds.	9.3 *Enterocytozoon hepatopenaei* risk assessment

Issue number	Issue	Department response	Final report reference
		The PLE of the hatchery exposure group to EHP did not change because it was assumed that a very small, yet significant volume of whole uncooked prawns could be used as feed for crustaceans in public aquaria and research facilities. The PLE for wild crustaceans was considered appropriate. The PLES for wild crustaceans was increased from **Low** to **Moderate** because a) EHP can remain infectious in the environment for extended periods, b) EHP infects commercially important prawn species present in Australia, c) EHP vectors are present in Australia (Artemia, crabs, oysters, polychaetes, squid) and d) prawns affected by EHP do not usually die which makes their predation by non-susceptible species less likely when compared to animals suffering acute disease. The overall impact estimated for EHP was determined to be appropriate considering the available information about its impacts overseas.	
51	Head and shell removal plus deveining does not address the risk of EHP in imported prawns	**Noted.** The risk assessment has been reviewed and updated according to new information. The PLES for wild crustaceans was increased from **Low** to **Moderate** because a) EHP can remain infectious in the environment for extended periods, b) EHP infects commercially important prawn species present in Australia, c) EHP vectors are present in Australia (Artemia, crabs, oysters, polychaetes, squid) and d) prawns affected by EHP do not usually die which makes their predation by non-susceptible species less likely when compared to animals suffering acute disease. To determine if the risk of EHP was managed by freezing, the department commissioned the University of Arizona to conduct independent research investigating the effect of commercial freezing on the infectivity of EHP in commodity prawns. Based on the results, it was considered that freezing played a significant role in managing biosecurity risks of EHP in imported prawns by reducing the likelihood of entry but not completely removing it. However, the overall annual risk was found to be **low** and as it does not achieve Australia's ALOP, specific biosecurity measures are considered necessary. The overall restricted risk of imported prawns with head and shell removal applied was determined to be **low**. This is because head and	9.4 *Enterocytozoon hepatopenaei* biosecurity measures

Issue number	Issue	Department response	Final report reference
		shell removal of frozen prawns is not expected to reduce the likelihood of entry. Whilst, head and shell removal would reduce the parasite load in the prawn, sufficient EHP to cause infection is expected to remain in the gastrointestinal tract. Therefore, deveining, and head and shell removal are necessary to manage the risk of EHP. Deveining is expected to reduce the load of viable EHP spores present in the imported prawn and therefore the likelihood of entry of EHP. The overall restricted risk of EHP in imported prawns with the biosecurity measure, head and shell removal plus deveining, applied was determined to be **Very Low**.	
-	Infectious myonecrosis virus	-	-
52	Review risk assessment for IMNV, given the likelihood of exposure, establishment and spread of IMNV and susceptibility of the known Australian species which are of significant commercial importance for both aquaculture and wild fisheries.	**Noted.** The PLE of farmed crustaceans to IMNV reduced from **Low** to **Very Low**. This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds. The PLE of the hatchery exposure group to IMNV did not change because it was assumed that a very small, yet significant volume of whole uncooked prawns could be used as feed for crustaceans in public aquaria and research facilities. The PLE of wild crustaceans to IMNV was considered appropriate due to the narrow host range of IMNV in Australia. The impact of IMNV was considered appropriate. Whilst *P. monodon* is susceptible to infection with IMNV, it is refractory to clinical disease and no mortalities have been reported in IMNV-infected *P. monodon*. *Penaeus merguiensis* and *Penaeus esculentus* are also susceptible to infection with IMNV and show some clinical signs due to infection, but no mortalities have been reported in these species.	10.3 Infectious myonecrosis virus risk assessment
53	Review the assessment for the PLES for IMNV.	**Noted**. The risk assessment for IMNV has been reviewed and the estimations for PLES were considered appropriate. The range of species susceptible to IMNV that are present in Australia is much narrower compared to some other hazards.	No change to the final report
-	Laem-Singh virus,	**-**	-

Issue number	Issue	Department response	Final report reference
54	The PLE, PLES and impacts for wild crustaceans were underestimated for LSNV The risk assessment needs to be reviewed.	The PLE of farmed crustaceans to LSNV reduced from **Low** to **Very Low**. This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds. The PLE of the hatchery exposure group to LSNV did not change because it was assumed that a very small, yet significant volume of whole uncooked prawns could be used as feed for crustaceans in public aquaria and research facilities. The PLE and PLES of LSNV for wild crustaceans were considered appropriate due to the narrow host range of LSNV in Australia. The overall impact estimated for LSNV was determined to be appropriate considering the available information about its impacts overseas.	11 Laem-Singh virus
-	Taura syndrome virus	-	-
55	Review the assessments for the PLE for TSV.	**Noted**. The PLE of farmed crustaceans to TSV in imported prawns reduced from **Low** to **Very Low**. This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds. The PLE of the hatchery exposure group to TSV did not change because it was assumed that a very small, yet significant volume of whole uncooked prawns could be used as feed for crustaceans in public aquaria and research facilities. The PLE of wild crustaceans to TSV was considered appropriate due to the narrow host range of species susceptible to TSV in Australia. The susceptibility of species in Australia, such as *P. monodon* and *P. merguiensis*, is dependent upon the method of exposure and the strain of TSV.	12 Taura syndrome virus risk assessment
56	Review the assessment for the PLES for TSV.	**Noted**. The risk assessment for TSV has been reviewed and the estimation for PLES was considered appropriate due to the narrow host range present in Australia. In addition, the susceptibility of species in Australia, such as *P. monodon* and *P. merguiensis*, is dependent upon the method of exposure and the strain of TSV.	No change to final report.

Issue number	Issue	Department response	Final report reference
-	*Vibrio parahaemolyticus* strains containing Pir toxins	-	-
57	The PLE, PLES and impacts for wild crustaceans were underestimated Vp AHPND. The risk assessment needs to be reviewed.	The department reviewed the PLE, PLES and impacts for Vp AHPND. The estimations for PLE and PLES ratings were considered appropriate. This is primarily due to the narrow host range, tissue tropism and impacts of freezing on Vp AHPND. The overall impact estimated for Vp AHPND was determined to be appropriate taking into account the available information about its impacts overseas.	13 *Vibrio parahaemolyticus* (strains causing acute hepatopancreatic necrosis disease) risk review
-	White spot syndrome virus	-	-
58	WSSV is present in Australia and therefore should not be retained as a hazard.	**Noted.** WSD is limited to an area in South-East Queensland which is under an official control program. WSD has also been detected at prawn farms on the NSW north coast, Clarence Valley. The farms were issued with a biosecurity direction by NSW DPI to carry out destruction of affected animals and decontamination of the premises. Surveillance is being conducted to determine if the virus has been eradicated from the farms and to determine if the virus is present in the immediate environment in the vicinity of the farms. At the time of preparing this report, there is no indication that the virus has spread beyond the affected premises. A control order is in place which restricts the movement of raw, uncooked decapod crustaceans from the Clarence Estuary while containment, source detection and surveillance activities are underway. Based on the situation and restrictions in both these areas, WSSV continues to meet the criteria for hazard identification listed by the WOAH Code.	No changes in final report
59	It is suggested that the most likely source of the WSD outbreak on the Logan River and Moreton Bay was via introductions of uncooked imported WSSV positive prawns being used as bait.	**Noted.** The source of the original WSD outbreak remains unknown. The department acknowledges that bait use is a possible pathway for introduction and the draft report adequately considers and highlights this. It is also stated that the most likely pathway by which wild prawns could be exposed to imported prawns is if they are used as bait or berley by recreational fishers.	1.5 White spot disease outbreak in Australia

Issue number	Issue	Department response	Final report reference
		A section regarding the white spot disease outbreak in Australia has been included in the final report.	
60	The PLE, PLES for wild crustaceans were underestimated for WSSV. The risk assessment needs to be reviewed.	**Noted**. The PLE of farmed crustaceans to WSSV reduced from **Low** to **Very Low**. This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds. The PLE of the hatchery exposure group to WSSV did not change because it was assumed that a very small, yet significant volume of whole uncooked prawns could be used as feed for crustaceans in public aquaria and research facilities. The PLE of wild crustaceans to WSSV was considered appropriate due to the broad host range of species susceptible to WSSV in Australia. The PLES of WSSV in wild crustaceans increased from **Moderate** to **High**. This is because of the detection of WSSV in wild crustaceans in Moreton Bay demonstrating that the hazard is more likely to spread due to its wide host range. The PLES of WSSV in farmed and hatchery crustaceans were considered appropriate.	14 White spot syndrome virus risk assessment
61	WSSV has been detected in wild commercial crustaceans from Moreton Bay and South-East Queensland. WSSV is now established in the region and environmental and economic impacts remain there permanently.	**Noted.** Information from the surveillance survey conducted in Moreton Bay and South-East Queensland has been added. The impact assessment for WSSV already considers that the impact is on-going.	14 White spot syndrome virus risk assessment
62	Risk assessment for WSSV needs to recognise that the consequences of exotic disease incursions are most likely to be extreme under most circumstances, as they erode Australia's economic competitiveness and threaten Australia's environment on a permanent basis.	**Noted.** The overall impact of establishment and spread of WSSV was estimated to be **High** and the overall annual risk was determined to be **Extreme**. When assessing impacts, the frame of reference is the impact of each hazard on the Australian community, rather than on the directly affected parties. For all hazards, including WSSV, the economic effects related to the costs associated with eradication, control, surveillance and monitoring, and compensation to regain a disease-free status was considered **Minor at the national level**. Which means the impact is	No change to final report

Issue number	Issue	Department response	Final report reference
		recognisable Australia wide, but the impact is also minor and reversible. A conservative approach was taken by the department when considering the susceptibility of native species, particularly those that are endangered or threatened, to infection with the hazards. Due to the wide host range of WSSV in Australia, which includes several decapod species listed as endangered, the effect of WSSV on the environment was also considered to be **Minor at the national level**. Which means the impact is recognisable Australia wide, but the impact is also minor and reversible. The department considers the impact of a WSSV outbreak have been adequately considered.	
63	Head and shell removal in combination with pre-export and on-arrival batch testing should reduce the risk for WSSV to Low (not Very Low) and therefore Australia's ALOP should only be achieved applying cooking.	**Noted.** The *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* report have become available since the release of the draft report. The survey showed that fishers are using cooked prawns for bait at a rate similar to that of uncooked shelled prawns. These usage patterns are not reflective of the assumptions in the draft report and therefore the exposure likelihoods for the WSSV risk assessment were revised. The department has determined that head and shell removal combined with pre-export and on-arrival batch testing would reduce the likelihood of entry of WSSV from **High** to **Extremely Low**. This is because of the testing program in place for imported prawns and reduced likelihood of prawns which have been deshelled and had the head removed being used as bait or berley. A new chapter has been included in the final report with detailed information regarding the testing program for imported prawns.	17 Testing of imported prawns
-	Yellow head virus genotypes 1	-	-
64	The PLE, PLES and impacts for wild crustaceans were underestimated for YHV1. The risk assessment needs to be reviewed.	**Noted**. The PLE of farmed crustaceans to YHV1 reduced from **Low** to **Very Low**. This is because of advice that broodstock and farmed prawns in Australia are not fed whole domestic or imported commodity prawns in hatcheries or in grow-out ponds.	15 Yellow head virus genotype 1 risk assessment

Issue number	Issue	Department response	Final report reference
		The PLE of the hatchery exposure group to YHV1 did not change because it was assumed that a very small, yet significant volume of whole uncooked prawns could be used as feed for crustaceans in public aquaria and research facilities. The PLE of wild crustaceans to YHV1 was considered appropriate due to the wide host range YHV1. The PLES of YHV1 in farmed and hatchery crustaceans were considered appropriate. The overall impact estimated for YHV1 was determined to be appropriate taking into account the available information about its impacts overseas.	
-	**Compliance activities**	-	-
65	The review does not consider inspection confusion, system rorting, testing failure, human error and deliberate criminal evasion.	**Noted.** Compliance issues are outside the scope of the review. The department utilises a range of verification activities to monitor system rorting. This includes border inspections, container compliance verification, the compliance-based interventions scheme (CBIS), random sampling of cooked prawn consignments, profiling and targeting of importers, document assessment and clearing procedures, non-compliance assessment and response, enforcement, and retail testing. As a result of this monitoring, a range of compliance and regulatory actions have been undertaken against importers since July 2017 when trade in uncooked prawns resumed. Actions include re-export and destruction of prawn consignments that failed on-arrival testing, suspension of approved arrangements in breach of requirements, suspension and refusal of prawn import permits and criminal convictions and fines for breaches of the Biosecurity Act 2015. The department has also undertaken targeted activities to ensure uncooked prawns are not being imported as other frozen foods, which was commended by the IGB in a review of the IGB report 2017. Verification and compliance activities for prawn imports are ongoing. Further information about these issues is presented in Chapter 17 and Appendix C.	17 Testing of imported prawns Appendix C Recommendations of the Inspector General of Biosecurity

Appendix C: Recommendations of the Inspector-General of Biosecurity's review of uncooked prawn imports and progress on their implementation

Background

Following the outbreak of white spot disease in south-east Queensland prawn farms in December 2016 and the suspension of uncooked prawn imports in January 2017, the Inspector-General of Biosecurity (IGB) undertook a review of the effectiveness of biosecurity controls implemented by the department for this commodity (IGB's review).

The scope of the IGB's review covered operational policy and activities relevant to biosecurity risks associated with importation of uncooked prawns and prawn meat into Australia. The IGB's review considered:

- the effectiveness of biosecurity controls and their implementation for managing the biosecurity risks of importation of uncooked prawns and prawn meat into Australia
- the effectiveness of post-entry surveillance measures and 'end use' import conditions for uncooked prawns and prawn meat into Australia
- areas for improvement in the biosecurity risk management framework and its implementation for future trade in prawns and related seafood.

The IGB *Uncooked prawn imports: effectiveness of biosecurity controls* report (IGB report 2017) was released on 12 December 2017 and can be accessed at the Inspector-General of Biosecurity website.

This appendix outlines the findings and recommendations of the IGB report 2017. A summary of the department's response and progress on the implementation of the recommendations is also provided.

Recommendations and progress on their implementation

Twenty of the 22 recommendations made by the IGB's review are now complete, with the release of this risk review as a final report, being the twenty first.

The IGB's review found several deficiencies in the management of the biosecurity risk of uncooked prawn imports, with broader implications for Australia's biosecurity risk management framework. The IGB report 2017 contained 22 recommendations to increase the effectiveness of biosecurity controls implemented by the department on uncooked prawn imports, to improve this biosecurity risk management framework and to improve its ability to deal with ongoing and emerging challenges.

The department agreed with the IGB's recommendations, either fully or in principle. The implementation of the recommendations is near completion, supported by ongoing business as usual activity, with some requiring decisions by third parties.

Table 23 lists the 22 recommendations in the IGB's report 2017 and a progress summary on the department's implementation and response to each recommendation as of 18 April 2023.

Table 23 Recommendations from the IGB's report 2017 and summary of implementation progress

Recommendation number	Recommendation	Progress summary
1	The department, in consultation with industry and state/territory governments, should review risk mitigation measures for the various pathways for white spot disease to enter and establish in Australia.	**Completed on release of this final report.** The department released the draft report for the *Review of the biosecurity risks of prawns imported for human consumption* for stakeholder consultation on 28 September 2020. The consultation period ended on 15 January 2021. The prawn review was subject to rigorous scientific review. This included the review of the draft and provisional final reports by the members of the scientific advisory group (SAG). On 30 April 2021, the department received the *Review of the draft report from the members of the SAG*. The department prepared a provisional final report considering the recommendations from the members of the SAG on the draft report, submissions received during the stakeholder consultation period, outcomes of the 2*019–20 National recreational fishing survey - prawn use by recreational fishers for bait and berley*, outcomes of research commissioned to investigate the effect of freezing and cooking on some prawn pathogens, and all other new information. On 3 November 2022, the department received the *Review of the provisional final report* from the members of the SAG. Their recommendations were considered in preparing this final report for public release.
2	The department should, as a priority, review the sampling regime for consignments of imported uncooked prawns and prawn products.	**Completed.** An improved sampling regime for consignments of imported uncooked prawns and prawn products has been implemented. The department released the draft report on 28 September 2020. The draft report did not propose changes to the improved sampling regime currently in place for consignments of imported prawns. Stakeholders had the opportunity to provide comment on the draft report until 15 January 2021. Comments received on the sampling regime will be considered when preparing the final report.
3	The department should facilitate research to validate the impact of cooking on white spot syndrome virus (WSSV) inactivation, and WSSV testing results.	**Completed.** The University of Arizona has completed research on WSSV inactivation during cooking. The department provided comment on the experimental design and endorsed the research proposal. The results of this experiment have been considered in the draft review. The same experiment showed that although cooking inactivates WSSV in prawns, testing of cooked prawns using accepted DNA detection methods returned positive results.
4	The department should review import conditions for uncooked prawns listed on the Biosecurity Import Conditions system (BICON) to ensure clarity and consistency with World	**Completed.** Import conditions have been reviewed and updated and will be further reviewed as the risk review progresses.

Recommendation number	Recommendation	Progress summary
	Organisation for Animal Health (WOAH, formerly OIE) terminology, scientific accuracy and usefulness for verification at the border.	
5	The department should work with competent authorities and industry to encourage the import of uncooked prawn products from specific pathogen-free countries, zones, or compartments. This should be industry-driven and involve: • quality-assured supply chain management • competent authority verification of pre-border status of consignments, and • regular departmental offshore audits or verifications of these arrangements.	**Completed.** The department undertook familiarisation visits to prawn exporting countries in 2017 and 2018 to discuss implementation of the enhanced import conditions, and to observe pre-border controls for prawns. In 2019, the department reviewed Malaysia's certification systems for uncooked prawns and New Caledonia's status as a country free of disease of biosecurity concern. As a member of the World Trade Organization, Australia has international obligations to consider trading partner requests for the assessment of equivalence in risk management measures, such as disease freedom. Assessment of equivalence requests for compartment, zone, and country freedom from diseases of biosecurity concern will recommence on completion of the prawn review.
6	The department should continue to conduct full seals-intact inspections of uncooked prawn imports (by at least two inspectors). It should also review measures to ensure integrity of the seals-intact containers until inspection.	**Completed.** The department now ensures two inspectors always undertake inspections. The work instructions require inspectors to check the integrity of the seal and main components of the door for tampering.
7	The department should implement and publicise an ongoing program of random and risk-based, seals-intact inspections of frozen goods to ensure that uncooked prawns are not being falsely imported as other frozen foods.	**Completed.** The department has undertaken ongoing targeted activities since the imported prawn suspension was lifted in July 2017.
8	The department should ensure that inspections at approved arrangements, especially at regulated cold stores, are periodically carried out by two inspectors. If only single inspectors are available, they should be regularly rotated.	**Completed.** The container must remain unopened and seals intact at the Approved Arrangement until a biosecurity officer is present. All uncooked prawn inspections must now be conducted by two biosecurity officers.
9	The department should facilitate the development and implementation of a nationally consistent competence and verification framework covering staff involved in assessing and inspecting imported uncooked prawns and other commodities. This should be regularly reviewed and adequately resourced.	**Completed.** The department has developed and implemented a competency framework for biosecurity officers. Protocols have been developed to ensure ongoing assessment of officers against the framework.

Recommendation number	Recommendation	Progress summary
10	The department should improve internal communication to develop and implement training processes, instructional material and work directions that are technically sound, suit the conditions being experienced and are applied as intended. These should be monitored and regularly reviewed.	**Completed.** Updated work instructions for assessing, inspecting and sampling of prawns and prawn products were published in August 2017. These documents are regularly reviewed and updated as required. The department has implemented improved internal communication arrangements, updated instructional material and has strengthened arrangements to ensure that the required processes are being applied as intended.
11	The Australian Government should commit to ensuring adequate long-term funding for biosecurity risk management, including border inspections and enforcement. Funding should be linked to growth in imports and biosecurity risks, with cost-recovered functions exempt from efficiency dividends and staff ceilings.	**Completed.** This is a matter for government. In responding to the 2017 independent review of the national biosecurity system, Australian agriculture ministers confirmed their support for ensuring the national biosecurity system is resourced to maintain an appropriate level of protection. The government approved additional resources in 2017-18 to manage the initial response to the WSSV prawn outbreak. This additional resourcing reduced over time as the intervention was integrated into normal operations. Funding has been secured through several budget processes to support strengthening the biosecurity system, most recently in the 2020-21 MYEFO. In 2020–21, around $873 million is available for biosecurity and export programs, representing an increase of $243 million since 2014–15. Biosecurity has received a significant funding boost through the 2021-22 Budget. The Government is investing AU$385.5 million for biosecurity systems, as well as AU$29.1 million in pest and weed reduction activities to help farmers manage threats. The Government is also funding cutting edge technologies for increased surveillance and targeting specific threats.
12	The department should consider seeking stronger powers under the Biosecurity Act 2015 to apply direct penalties for serious non-compliance and impose administrative sanctions or on-the-spot fines for relatively minor non-compliance.	**Completed.** The Biosecurity Amendment (Traveller Declarations and Other Measures) Act 2020 commenced on 1 January 2021. It enables the department to issue infringement notices for up to 12 penalty units (AU$2,664) to incoming travellers who fail to declare specified high-risk goods (including raw or partially raw prawns) – an increase from 2 penalty units (AU$444). The Biosecurity Amendment (Strengthening Penalties) Act 2021 came into effect on 30 June 2021 and increased a number of civil and criminal penalties under the Biosecurity Act. The department is continuing to review penalties under the Biosecurity Act. Further legislative amendments regarding penalties include proposed civil and criminal penalty increases included in the Biosecurity Amendment (Enhanced Risk Management) Bill 2021 that was introduced to parliament on 1 September 2021.

Recommendation number	Recommendation	Progress summary
13	The Director of Biosecurity should seek powers under the Biosecurity Act 2015 to conduct a general recall of goods for biosecurity purposes.	**Completed.** Legislation to provide the Director of Biosecurity with appropriate secure and advise powers received Royal Assent on 31 August 2018.
14	The department should continue to improve internal biosecurity risk governance and communication to rapidly identify emerging biosecurity risks. Risks should be communicated to governments, the wider community and industry through a defined and documented triaging and escalation procedure.	**Completed.** Internal biosecurity risk governance is being improved, including the establishment of an active risk management group. Further review of governance is underway.
15	The department should discuss with the National Biosecurity Committee mandatory reporting of all post-border detections of prescribed exotic disease agents or pests to Australian and state/territory government departments.	**Completed.** Protocols were finalised and signed at the National Biosecurity Committee (NBC) meeting in June 2018.
16	The department should collaborate with state/territory agencies, Animal Health Australia and relevant industry bodies to review and implement more effective communication policies to aid the early dissemination of information about exotic aquatic diseases and pests and their management to stakeholders.	**Completed.** Processes to improve communication about heightened disease risks with state and territory jurisdictions are being implemented. This includes the confidential communication of critical biosecurity investigations. Draft protocols to share biosecurity data and information between the Commonwealth and States and Territories have been developed in consultation with the states and territories. The protocols were finalised and signed at the National Biosecurity Committee meeting in June 2018.
17	The department should formalise, oversight and monitor stronger prescribed arrangements for laboratories undertaking import testing to ensure their accountability and ongoing implementation of prescribed testing standards.	**Completed.** The department has strengthened the arrangements for oversighting and monitoring the performance and accountability of approved laboratories. A standardised WSSV test procedure was implemented at all approved laboratories in October 2017. Quality assurance testing for WSSV in approved testing laboratories is conducted 3 times a year. Laboratory approved processes have been updated and a new class of Approved Arrangement (AA) specifically for imported prawn testing has been developed. Implementation of the new AA class will occur once the Australian Centre for Disease Preparedness (ACDP)' WSSV testing manuscript has been published in a peer reviewed journal.
18	The department should, in collaboration with National Association of Testing Authorities, oversight the performance of import testing laboratories in quality assurance programs. This should include regular proficiency testing and assessment of control samples distributed among the laboratory network, with	**Completed.** The department finalised and approved a quality assurance program for WSSV testing of imported prawns on 20 March 2018. In collaboration with the ACDP, the department has implemented 8 rounds of this program. To date, all approved laboratories have demonstrated acceptable results.

Recommendation number	Recommendation	Progress summary
	means to ensure that laboratories rectify any identified deficiencies in a reasonable period of time.	
19	The department should promote an update of the old Australian and New Zealand standard diagnostic procedure (ANZSDP) for WSSV including peer review of the new Procedure for detection of WSSV for biosecurity risk management. This should be conducted by a suitably resourced national technical group formed from the Animal Health Committee's (AHC) National Laboratory Task Group and Sub-Committee on Aquatic Animal Health (SCAAH).	**Completed.** The research work has been completed by scientists at ACDP. The research paper detailing the updated procedures has been published. The updated ANZDP for WSSV was published in February 2023.
20	The department should maintain strong links with aquatic health and production experts in Australian and state/territory government agencies, universities and industry, to support decision-making based on: • the latest scientific knowledge of new technologies, and • international emergence, movements and risks to Australia of serious aquatic animal diseases.	**Completed.** The department held a roundtable discussion of the review process with key stakeholders in Canberra on 8 February 2018. The department sought submissions from stakeholders on Australia's current import policy for prawns on 26 March 2018. The hazard table was subject to independent peer review, and stakeholders had the opportunity to comment on it when the Draft prawn review report was released for stakeholder consultation in September 2020. The Draft prawn review report was subject to independent peer review and comment by stakeholders. A webinar was held in November 2020 to discuss the outcomes of the draft report. The department also intends to use the Scientific Advisory Group during the review process. The department will take into account current scientific information when completing the review. The department monitors international disease alerts and reports to keep up-to-date with emerging disease risks. New scientific information can be provided to the department at any time, including after a risk analysis has been completed.
21	The department should continue to work with Animal Health Australia, state/territory agencies and aquatic industries to develop an aquatic emergency animal disease response agreement (deed) as soon as possible.	**In progress.** Development of the Aquatic Deed is well progressed. A complete draft has been legally reviewed and prospective parties have provided comments.
22	The department and state/territory governments and industry should agree on (and cost share, as relevant) measures for monitoring and minimising risks of any imported uncooked prawn product entering waterways. Measures could include:	**Completed** The department has undertaken numerous activities in response to this recommendation. Including surveillance of retail prawns for WSSV, undertaking a recreational fishing survey to gather current national data on the use of prawns as bait or berley by recreational fishers and

Recommendation number	Recommendation	Progress summary
	• periodic surveillance of retail prawns for target diseases • periodic assessment of fishing practices • targeted public awareness programs discouraging use of imported prawns as bait • prevention of recreational fishing and surveillance of wild crustaceans close to prawn farms, and • surveillance of bait shops to ensure they are not selling prawns imported for human consumption.	targeted programs to raise awareness about the biosecurity risks associated with the importation of aquatic animals (including prawns).

Appendix D: General considerations

This chapter provides details on the general considerations taken into account by the department when undertaking this risk review. Where relevant, explanation is provided for changes in assumptions or conclusions between this risk review and the *Generic import risk analysis report for prawns and prawn products 2009* (Prawn IRA 2009) (Biosecurity Australia 2009).

Entry assessment

The World Organisation for Animal Health (WOAH, formerly OIE) *Aquatic animal health code* (WOAH Code) (WOAH 2022c) describes the entry assessment as:

> The biological pathway(s) necessary for an importation activity to introduce a pathogenic agent into a particular environment, and estimating the probability of that complete process occurring, either qualitatively (in words) or quantitatively (as a numerical estimate).

The entry assessment determines the annual likelihood of entry into Australia of each hazard. In this risk review, consideration is given to the single-entry scenario which is the importation (from all countries) into Australia of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption (imported prawns). It is considered that this commodity type represents the highest biosecurity risk.

Figure 8 depicts the key points in the prawn import pathway that make up the single-entry scenario, from sourcing prawns from farms in the exporting country, through to the first point of entry into Australia (assuming no import conditions are in place to manage biosecurity risks).

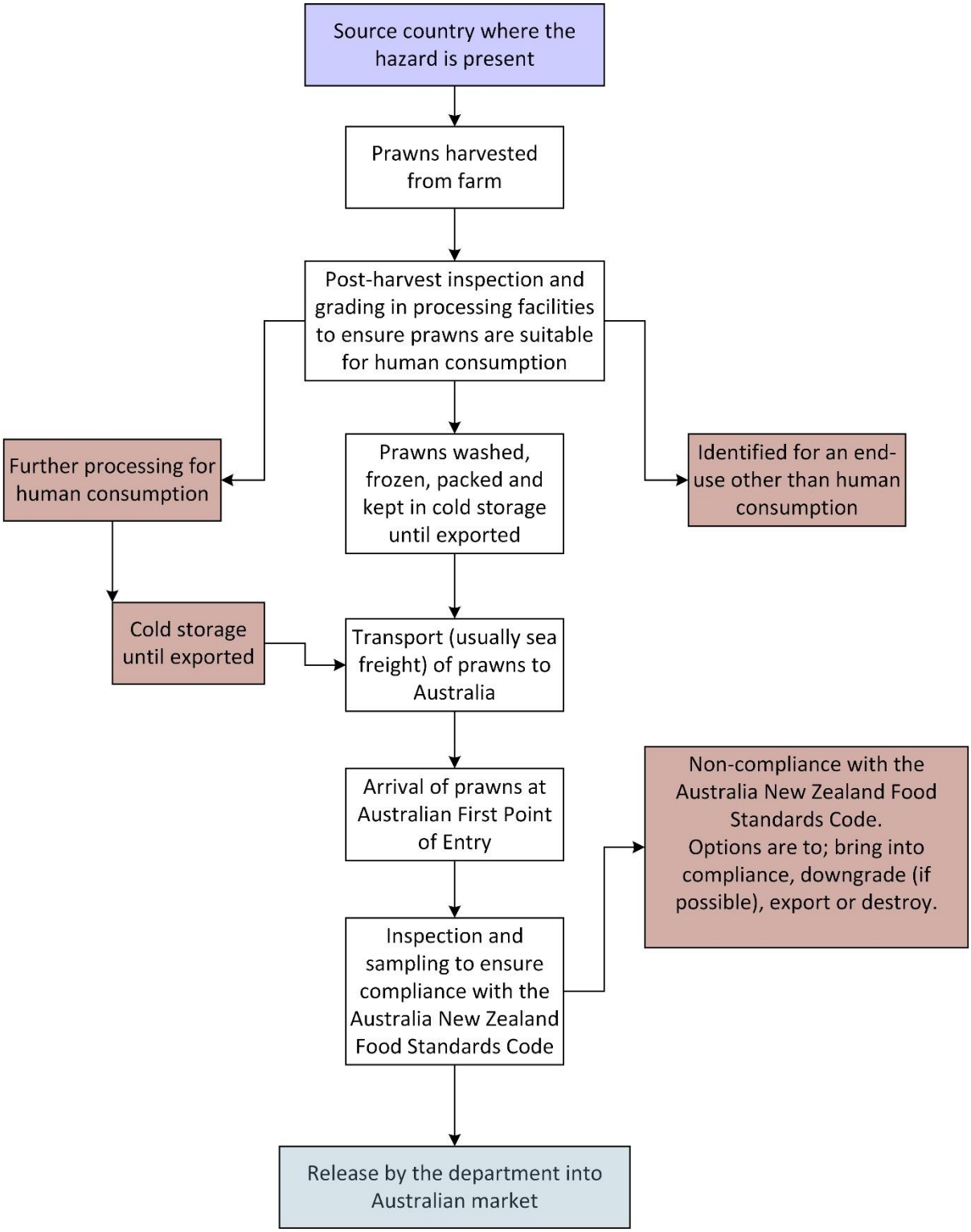
Almost all imported prawns arrive in Australia as commercial sea cargo in 20 or 40 foot refrigerated containers (that is, approximately 750 to 1500 cartons per consignment, with some consignments weighing up to 20 tonnes). Biosecurity officers responsible for inspecting imported prawn consignments report that almost all product is individually quick frozen (IQF), with only an occasional product imported in the form of 1kg frozen blocks (Personal Communication, Department of Agriculture, 2019).

Three key factors are relevant in determining the likelihood of viable and infective hazards being present in prawns imported into Australia. These key factors are:

- biological characteristics of the hazards in harvested prawns
- likelihood of detection and removal of infected prawns by post-harvest or on-arrival inspection
- ability of the hazard to remain infectious through processing, transport and storage.

The absence of a pathogenic agent from a region is also an important consideration in an entry assessment. However, as the scope of this risk review includes importation of prawns from all countries, the entry assessment assumes that the hazards are present in all source countries. Country, compartment or zone freedom from hazards are considered as a biosecurity measure during risk evaluation (although, they are not considered specifically in each risk assessment, see section 5.1.1 Sourcing from free populations).

Figure 8 Entry pathway for unrestricted import of prawns into Australia



The key points in the prawn import pathway, from sourcing prawns from farms in the exporting country, through to the first point of entry into Australia. This diagram assumes no import conditions are in place to manage biosecurity risks and therefore there is no biosecurity intervention before release into the Australian market.

Key factors considered in entry assessment

Biological characteristics of the hazard in harvested prawns

There are several biological factors of the hazards that are considered during the entry assessment.

Species of prawn

Some pathogenic agents infect a wide range of species. For example, white spot syndrome virus (WSSV) can infect multiple crustacean species (OIE 2021k). Other pathogenic agents are generally more restricted in host range, such as infectious myonecrosis virus (IMNV), which only infects certain *Penaeus* species (OIE 2021g).

Life-cycle stage

The prevalence of infection and/or the expression of disease may vary with the life-cycle stage of the host. For example, yellow head virus genotype 1 (YHV1) outbreaks are most common when prawns are in the juvenile to sub-adult stage (Lightner 1996b). Also, infection with Taura syndrome virus (TSV) appears to have no impact on nauplii, mysis and early postlarval (PL) stages, but may exhibit as disease in prawns from about PL12 onwards (Brock 1997a; Lightner 1996b). Prawns that survive disease outbreaks can become reservoirs of infection in later life-cycle stages. Such is the case with YHV1 and IMNV, where adult prawns can survive and remain infectious without showing clinical signs of disease (Anantasomboon et al. 2008b; Boonyaratpalin et al. 1993; Cowley et al. 2011; Lightner et al. 2004; Srisala et al. 2020a; Tang et al. 2005).

Tissue tropism and infectious dose

Tissue tropism reflects the ability of a pathogenic agent to infect a specific tissue type or location in the host. For example, some pathogenic agents can infect numerous tissues and organs, while others are restricted to a specific tissue type or location in the host. The tissue tropism of a pathogenic agent has the potential to affect the likelihood of entry because, for example, removal of the shell or gut from whole prawns would reduce the number of organisms that are preferentially located in those regions.

Prawn heads are likely to have high titres of pathogenic agents that have a preference for the hepatopancreas (for example, *Vibrio parahaemolyticus* strains containing Pir toxins (Vp AHPND) (OIE 2021b). Shell or head removal would not significantly reduce the load of pathogenic agents such as IMNV that are preferentially located in muscular tissues (Tang et al. 2005).

Infection by bacterial or viral pathogenic agents may result in bacteraemia or viraemia, and as a result, the pathogenic agent will be present throughout haemolymph rich tissues. In such cases, the removal of the head would reduce the amount of the pathogenic agent but would not eliminate it from the rest of the animal. The viral load in the haemolymph of *Penaeus vannamei* infected with decapod iridescent virus 1 (DIV1) is up to 110 times higher when compared to DIV1 load in muscle (reported to contain the lowest DIV1 loads) (Qiu et al. 2018a). Removing the head of DIV1 affected prawns would be expected to significantly reduce the DIV1 load.

In some cases, removal of the tissue carrying the highest pathogenic agent load may still leave a dose sufficient to cause infection in a susceptible host animal, should there be an exposure. For example, removal of the head of WSSV-infected prawns may only reduce the viral load by approximately half. Experimental infections in *P. vannamei* found that on a per weight basis, 49% of the WSSV viral load was in the head of the prawn (2.00×10^{10} WSSV copies/g tissue) and 51% in the whole tail (shell and

meat) (1.53×10^{10} WSSV copies/g tissue) (Durand et al. 2003). It was further extrapolated that the meat portion of the tail would be expected to contain 45% of the viral load of the whole tail (Durand et al. 2003). It is expected that the viral load that would remain in a WSSV-infected prawn tail if the head, or head and shell, was removed would be sufficient to cause infection in a susceptible species. For example, 2000 copies of WSSV genome resulted in a cumulative mortality greater than 80% 14 days post-exposure (Gitterle et al. 2006). Removing the head may also slow the enzymatic degradation of any remaining pathogenic agent present in the prawn causing it to persist for longer (Bondad-Reantaso, Tran & Thi Thanh Hue 2013).

Subclinical or chronically infected prawns and recovered prawns may not contain high concentrations of pathogenic agents throughout the body. The pathogenic agents may be concentrated in particular tissues such as the lymphoid organ, as is the case with TSV (Hasson et al. 1999) and YHV (Boonyaratpalin et al. 1993).

Production system

The production system, husbandry techniques and health management employed on-farm can have a profound influence on the health status of prawns. Prawns produced in extensive systems with low stocking densities typically have a lower prevalence of disease. This is presumably due to less efficient transmission of pathogenic agents and greater resistance to infection due to lower stress levels. Intensive culture systems require a much higher level of management to maintain productivity. For example, in an experimental WSSV infection, higher mortalities occurred in *Penaeus japonicus* reared at higher densities compared to lower densities. The variation in mortality was attributed to the higher opportunity for horizontal transmission of the virus when prawns were stocked at higher densities (Wu et al. 2001).

Likelihood of detection and removal of infected prawns by post-harvest or on-arrival inspection

Post-harvest inspection and grading

Industry employees in the exporting country primarily inspect prawns to verify that they are fit for human consumption. Inspectors conduct an organoleptic (touch, smell, visual) assessment, that allows abnormal prawns (for example, those with a loose, limp cephalothorax, discolouration, visible lesions or physical damage) to be identified. Grossly abnormal prawns are usually diverted for further processing or moved into the bait and pet food supply chains. Prawns downgraded for aesthetic reasons are often further processed by cooking to ensure consumer acceptance.

Prawn processing lines usually operate at high speed, allowing little time for detailed inspection. Under normal commercial arrangements, inspection and grading decisions are made at multiple points along the processing line. Trained employees detect prawns that do not meet specified criteria, which are usually simple and clear-cut (for example, no visible lesions and normal clean colour). Inspection and grading can result in the removal of many animals of abnormal appearance and thereby contribute to the reduction of biosecurity risk.

Under the current import conditions, competent authorities (CA) are required to attest that the exported uncooked prawns are free from visible signs of infectious diseases. Government officials do not necessarily inspect every consignment and instead may rely upon various certification and approval systems that are in place for approved exporters. Establishments seeking to export prawns must usually meet several requirements to qualify for an export health certificate. This most often means certification by an official certification body, who may assess and provide HACCP (hazard

analysis and critical control point) certificates, and registration and approval by the relevant CA. The CA audits production establishments (that is, farms, processing facilities), to ensure all certification pre-requisites are met, including an established HACCP system for food safety, traceability system, internal and external audits, sampling and laboratory testing to support claims of disease freedom (Hutchings & Breen 2002; Tookwinas & Keerativiriyaporn 2004).

HACCP systems are based on the monitoring of key (critical control) points in the production process to verify that the system is operating within defined food safety standards and that action is taken to detect and correct deficiencies, including in the management of 'failed' product. Such systems have largely replaced the traditional approach, which relied on inspection of the end-product to determine compliance with product safety and quality parameters. HACCP systems provide a structured approach to the control of key processes, such as operational hygiene and refrigeration. These key processes minimise potential problems with food safety and quality failures. HACCP systems emphasise early detection and prevention of undesirable practices (such as cross contamination between cooked and raw product) that are important to food safety and biosecurity risk.

On-arrival food inspection scheme

Food sold in Australia (whether domestically produced or imported) must comply with the Australia New Zealand Food Standards Code (FSC), developed by Food Standards Australia New Zealand (FSANZ), and the Country of Origin Food Labelling Information Standard 2016. The Imported Food Control Act 1992 and its subordinate legislation (the Imported Food Control Order 2019 and Imported Food Control Regulations 2019) establishes the imported food inspection scheme (IFIS) and sets the compliance requirements for imported food to meet Australian food standards.

Under the IFIS, food is referred for visual and label inspection and may also be sampled for analytical testing. Imported food is referred to the IFIS based on its risk to public health. FSANZ provides food safety risk advice to the department on whether a food poses a medium or high risk to public health. If a food poses a medium or high risk the department then classifies this food as risk food which is referred, inspected and sampled initially at a rate of 100%. Food considered to pose a low risk to public health is classified as surveillance food and is monitored for compliance with the FSC at a rate of 5%. Currently FSANZ classify imported cooked prawns as risk foods and imported uncooked prawns as surveillance foods.

The number of tests that are applied to imported cooked prawns were amended in November 2020. The department has received risk advice from FSANZ that imported cooked prawns are a risk food for *Listeria monocytogenes, Vibrio cholera* and *Salmonella*. Testing for *L. monocytogenes* applies to cooked crustaceans that are ready-to-eat at a rate based on the compliance history of the food. Testing for *Salmonella* applies at the rate of 5%. Surveillance tests for nitrofurans and fluoroquinolones also applies. The products are tested as shown in Table 24.

Table 24 The imported food inspection scheme requirements for imported prawns

Product	Risk tests	Surveillance tests
Uncooked prawns	na	Nitrofurans[a] Fluoroquinonolones[a] Label and visual assessment

Product	Risk tests	Surveillance tests
Cooked prawns	*Listeria monocytogenes*	*Salmonella*
	Vibrio cholerae	Nitrofurans[a]
	Label and visual assessment	Fluoroquinolones[a]

na Not applicable; **a** farmed or aquaculture sources only

The referral rate applied to risk foods reduces as a compliance history is established between the producer, country of origin and tariff codes. After 5 passes, risk food is referred to IFIS at a rate of 25%. After an additional twenty passes the risk food is referred to the IFIS at a rate of 5%. If the risk food fails at any time the compliance history is removed. Risk food is not released until test results are assessed and the goods have passed testing. Surveillance foods can be released after the department's initial inspection. Importers and state food safety authorities are notified when a surveillance food fails analytical testing so post-border intervention can occur. Information about IFIS is on the department's website.

Ability of the hazard to remain infectious through processing, transport and storage

The conditions during processing, transport and storage of prawns can affect the persistence and therefore the likelihood of entry of an infectious pathogenic agent. Prawns for human consumption are typically packaged and stored after sorting, washing and freezing (see Post-harvest inspection and grading). Some prawn products may be stored and transported chilled, however Australia does not receive chilled uncooked product.

Washing

Processing procedures will vary considerably depending on the facility, however all prawns are expected to undergo washing in some form. It is more common to wash prawns using a water bath rather than a pressurised system.

Washing will likely reduce the amount of organisms located on the shell. HACCP procedures usually specify that water used in food-processing plants contain levels of residual chlorine that would contribute to the inactivation of any bacterial pathogens on the product. In most developed countries, human health authorities require the use of potable water in land-based food-processing plants. The water would usually contain a minimum residual level of 0.2 to 0.5 mg/L of free chlorine. The World Health Organisation reports that chlorine is present in most drinking water at a concentration of 0.2 to 1 mg/L (WHO 2003). However, some prawn pathogens would be unaffected by this concentration of chlorine and only those pathogenic agents on the external surfaces of the prawns would be exposed to the water.

Washing may also facilitate contamination, that is, the transfer of a pathogenic agent within and between processing runs (or batches). The significance of such transfer will vary with the agent under consideration. For example, pathogenic agents for which the expected prevalence between and within batches is already high, the transfer of the pathogenic agent in water baths is not likely to significantly alter any evaluations made.

Cold storage and transport (chilled)

Most viruses of aquatic animals will remain viable at chilled temperatures for hours to days, whilst bacteria which are pathogenic (or potentially pathogenic) to aquatic animals are generally inactivated to some degree by chilled storage (ADVS 1999). For example, some *Vibrio* species are

sensitive to refrigeration (OIE 2021b). It is unknown whether the strain causing acute hepatopancreatic necrosis disease (AHPND) is similarly affected.

Frozen storage and transport

Most prawns imported into Australia are frozen. Frozen prawns intended for human consumption are transported at a temperature of less than –18°C, and may be held in frozen storage for many months (ADVS 1999).

Storage at freezing temperatures kills many food-borne pathogenic protozoa, cestodes and nematodes (Archer 2004), but most viruses are stable at freezing temperatures (Hasson et al. 1995; Lightner et al. 1997b; Lu et al. 1995). Diagnostic and research laboratories commonly freeze prawn samples to ensure the preservation of viruses. Under laboratory conditions, maximum preservation of viral infectivity is achieved when samples are held at very low temperatures (– 70°C or lower). Bacteria that are pathogenic or potentially pathogenic to aquatic species are often inactivated to some degree by freezing (ADVS 1999; Su & Liu 2007). For example, transmission of Vp AHPND, was not possible from frozen prawns (Tran et al. 2013a).

Repeated freezing and thawing may also reduce the viability of some pathogenic agents, whereas others are not affected. For example, TSV reportedly survives multiple freeze-thaw cycles in prawn tissues (Hasson et al. 1995). Whereas, *Photobacterium phosphoreum* which has been isolated from prawn hatcheries presenting luminous bacterial diseases, is extremely susceptible to freezing and can be eliminated after a single freeze–thaw cycle (Archer 2004; Emborg et al. 2002). Because repeated freezing and thawing is likely to affect the quality of the product, it is unlikely to occur as a normal processing or storage step.

Multiplication during storage

In considering the effect of storage (both frozen and chilled) on microorganisms in or on food, it is important to note that viruses and parasites cannot multiply in food as they require live host cells to replicate (USDA 2012) and therefore the amount of these hazards will not increase during storage. Conversely, many bacteria are capable of replicating in food product over time. Although, this is more likely to be associated with products being kept at higher temperatures than would be acceptable for prawn products. For example, *V. parahaemolyticus*, can multiply on seafood at temperatures above 10°C (FAO & WHO 2011; Thomson & Thacker 1973; Vasudevan et al. 2002). Whether there is any potential for bacteria of biosecurity concern to increase in dose in prawn products during storage is unknown. However, it is unlikely given it would be expected that commensal organisms and environmental bacteria are likely to multiply much more rapidly and would effectively overgrow any aquatic pathogens present in the tissues. It would also be most likely that such temperature abuse would result in prawn tissues rapidly deteriorating and being unacceptable for human consumption.

Exposure assessment

The exposure assessment determines the likelihood of direct exposure of a susceptible population (exposure group) in Australia to each hazard via potentially infected imported prawns (or associated wastes). The exposure assessment does not consider exposures such as farmed crustaceans exposed to infected wild crustaceans. This exposure is considered when determining the likelihood of establishment and spread during the consequence assessment (see section 4.5 Consequence

assessment). All estimates of the likelihood of exposure assume the hazard is present in the imported prawns at the time of arrival in Australia.

The factors considered when estimating the likelihood of an exposure group encountering a hazard, for each major exposure pathway (from entry into Australia, through storage, transport, end-use and any associated waste disposal), included the:

- likelihood of imported prawns (or associated wastes) entering the general environment of the exposure groups
- amount of infectious hazard in imported prawns (or associated wastes) at point of exposure
- contact between susceptible host animals and imported prawns (or associated wastes).

Identification of exposure groups

The three exposure groups considered in this risk review are:

- farmed crustaceans
- hatchery crustaceans (encompassing crustacean hatchery broodstock and postlarvae as well as crustaceans in research facilities and public aquaria)
- wild crustaceans.

Except for the covert mortality nodavirus (CMNV) risk assessment (refer chapter 7 Covert mortality nodavirus risk review), these three exposure groups remain unchanged from the Prawn IRA 2009.

Identification of exposure pathways

The exposure assessment considers the key distribution pathways and end-uses that may result in the three exposure groups encountering each hazard.

Prawns imported for human consumption may be sold to consumers, become waste or be diverted to other uses. Exposure pathways that are direct and that have a high probability of completion contribute substantially to the total likelihood of exposure occurring (for example the use of prawns as bait or berley for recreational fishing).

The Prawn IRA 2009 considered that the majority of prawns imported for human consumption (and purchased as seafood) would be 'used' in one of three ways:

- consumption by humans
- disposal to a municipal garbage system
- used as bait or berley.

For the purposes of this risk review these assumptions are still considered valid.

The Prawn IRA 2009 identified that prawns purchased as seafood might be used or discarded in other ways, such as the:

- deliberate feeding of seabirds
- 'disposal' of uncooked prawn waste from picnics and other outdoor events to open areas where they might be accessible to scavengers such as seabirds
- direct use (whether deliberate or inadvertent) in aquaculture ponds.

The Prawn IRA 2009 incorporated these three potential pathways into the 'use of imported prawns as bait or berley for recreational fishing' because it was assumed that a comparatively low volume of commodity would be used or discarded in this manner. However, for this risk review it is considered that 'direct use (whether deliberate or inadvertent) in aquaculture ponds' would be more appropriately captured under the major exposure pathway Use of imported prawns as feed for crustacean broodstock and crustaceans in research facilities and public aquaria. The Prawn IRA 2009 identified that conditioning and feeding of crustaceans is not limited to the hatchery or farm setting. Fresh seafood is a primary dietary component for feed used in research facilities, teaching institutions and public aquaria throughout Australia (Biosecurity Australia 2009).

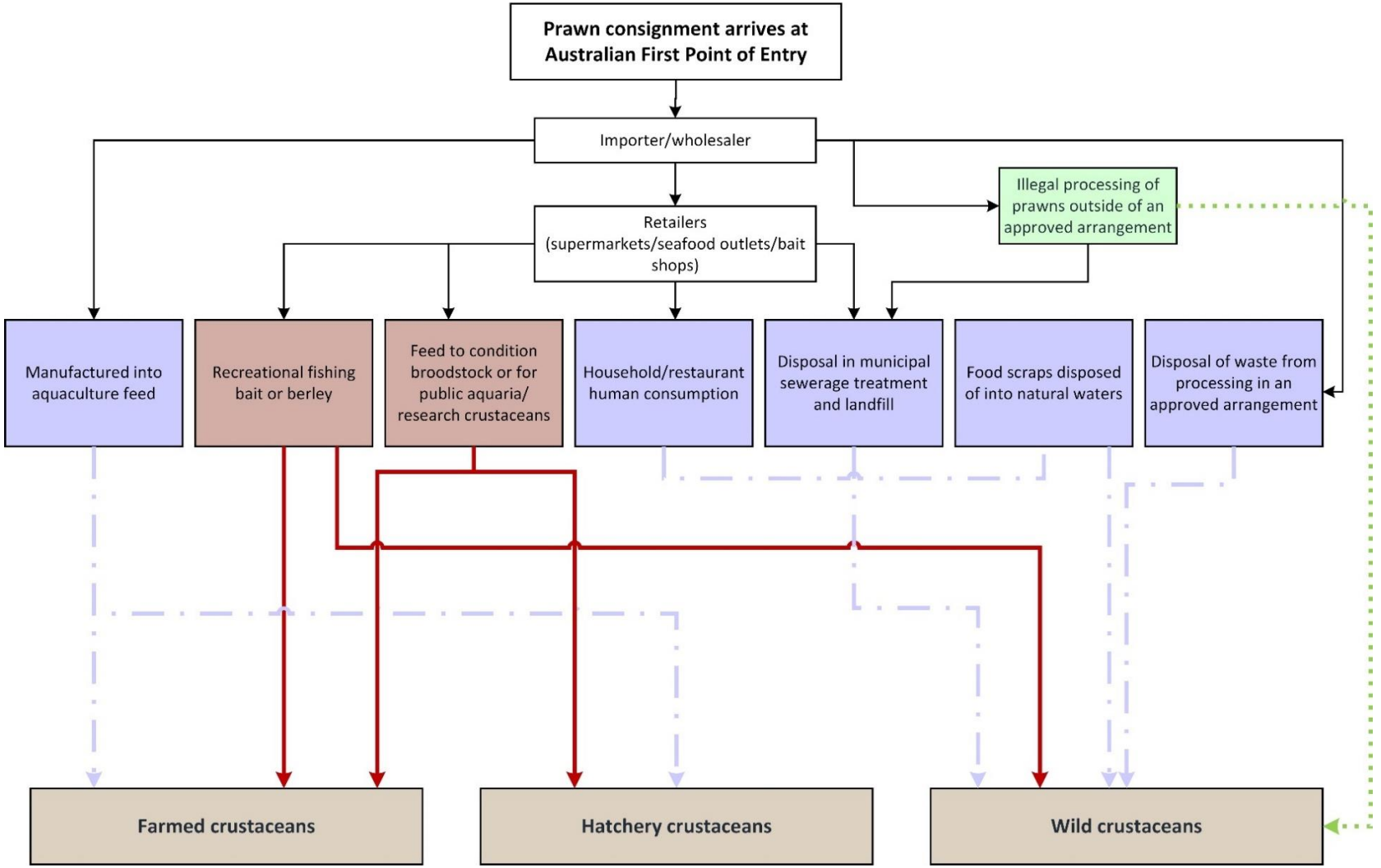
The (major) pathways identified as substantially contributing to the total risk were:

- Use of imported prawns as bait or berley for recreational fishing.
- Use of imported prawns as feed for crustacean broodstock and for crustaceans in research facilities and public aquaria.

The Prawn IRA 2009 included a third pathway in the 'major exposure pathway list'; 'disposal of solid and liquid waste from commercial processing of imported prawns'. This is no longer considered a major exposure pathway because commercial processing of whole, uncooked imported prawns is not permitted. Uncooked prawns which have had the head and shell removed and which do not meet import requirements on-arrival would not be permitted to be processed (for example, cooked) unless within an approved arrangement under agreement by the department, and would otherwise be directed for export. Additionally, breaded, battered and crumbed prawns must be sold in their imported form and must not be altered in any way, further processed or repackaged without written approval from the department. Approval to conduct any of those activities would only be given if the activity could occur in an approved arrangement. The Disposal of solids and liquid waste from commercial processing of imported prawns further outlines the considerations for this pathway (see Appendix E).

The Prawn IRA 2009 identified several minor exposure pathways. These exposure pathways have a much lower probability of completion because inactivation of the hazard occurs before potential exposure or they involve only indirect exposure of the aquatic environment. These pathways are discussed (see Appendix E) but were not considered further when conducting the risk assessments for this risk review. Figure 9 depicts the most likely (major and minor) pathways by which the three exposure groups could be exposed to imported prawns in Australia.

Figure 9 Potential exposure pathways of susceptible populations in Australia to imported prawns



Major exposure pathways that substantially contribute to total risk are dark red boxes and solid red lines (see Major exposure pathways). Minor exposure pathways are light purple boxes and purple dash-dot lines (see Minor exposure pathways in Appendix E). The green box and green dotted line are associated with an illegal pathway (not within the scope of this risk review).

The Prawn IRA 2009 also considered ballast water discharge, biofouling of vessels and the import of other aquatic animal commodities as potential exposure pathways by which some pathogenic agents associated with imported prawns may also be introduced into Australia. It was determined that those exposure pathways were outside of the scope because they were not associated with prawns imported for human consumption. This decision also applies to this review. However, it is highlighted that the risks associated with these pathways have been considered through other processes to minimise biosecurity risks to Australia. For example, all vessels operating internationally and domestically in Australia are required to manage ballast water. Ballast water is regulated under the Biosecurity Act 2015 along with its subordinate legislation the Biosecurity (Ballast Water and Sediment) Determination 2017 and the Biosecurity (Ballast Water Same Risk Area) Instrument 2017.

Likelihood and amount of imported prawns (or associated wastes) entering the general environment of the exposure groups

The likelihood and amount of imported prawns (or associated wastes) entering the general environment of the three exposure groups was considered for the pathways that substantially contribute to the total risk. There are two major exposure pathways identified in this risk review (see Figure 9):

1) Use of imported prawns as bait or berley for recreational fishing.
2) Use of imported prawns as feed for crustacean broodstock and crustaceans in research facilities and public aquaria.

The Prawn IRA 2009 considered that the minor exposure pathways (see Appendix E) were unlikely to add significantly to the overall risk and that any biosecurity measures required to manage the major exposure pathways were likely to be sufficient to mitigate the minor pathways. This conclusion is still valid, and the minor exposure pathways are not considered further in this risk review.

Major exposure pathways

1) Use of imported prawns as bait or berley for recreational fishing

The use of prawns as bait or berley poses a disease risk to susceptible species if the prawns are not intended for use as bait, that is, if they are intended for human consumption. This is especially the case for prawns which have been imported and potentially carrying exotic pathogenic agents. The Prawn IRA 2009 considered that the regular introduction of imported prawns, intended for human consumption, into the aquatic environment through use as bait or berley presented a significant pathway for exposure of wild crustaceans to imported prawns potentially infected with hazards. This conclusion is still considered to be true for the purposes of this risk review.

Surveys conducted by Kewagama Research in 2002 and 2007 investigating the use of prawns, intended for human consumption, as bait or berley provided significant data inputs for the exposure assessment and when considering biosecurity measures in the Prawn IRA 2009. The *National survey on bait-use by recreational fishers* (2002 survey) involved a random sample of 8000 households across Australia, with 1,123 fishers surveyed in detail (Kewagama Research 2002). The fishers questioned in the *National bait and berley follow-up survey, a follow-up survey to the National bait and berley survey 2002* (2007 survey) were composed of the original respondents from the 2002 survey. By interviewing those respondents, a 'before and after' assessment could be undertaken. This group was termed the 'repeat fisher group'. The 'repeat fisher group' represented 33% of total fishers from the 2002 survey (Kewagama Research 2007).

Prawns were a preferred option for recreational fishers with 62.6% of recreational fishers reporting use of prawns in a 12 month period in 2002 (Kewagama Research 2002). In 2017, Biosecurity Queensland commissioned Kantar Public to gather information about awareness, attitudes, beliefs and behaviours around white spot disease (WSD) amongst recreational fishers in Queensland (Kantar Public 2017). Data from the survey conducted by Kantar Public reported that plastic lures, uncooked prawns and caught fish were the most common baits used by respondents (Kantar Public 2017). A total of 1001 recreational fishers living in Queensland, and that indicated they fished within the last 12 months, participated in the online Kantar Public survey.

The *2019–20 National recreational fishing survey–prawn use by recreational fishers for bait and berley* (2019–20 Bait and berley survey) reported that nationally, the top five bait types in order of usage, were prawns (42.5%), other saltwater fish (38.7%), cephalopods (34.8%), saltwater worms (24.4%), and other shellfish (18.9%) (Moore et al. 2022). It is important to note that there was a total of 23,747 participants in the 2019–20 Bait and berley survey -online component. From the 23,747 participants, 17,596 responded to questions regarding their fishing participation. A subset of 11,849 participants indicated they fished within the last 12 months. A further subset of 5,514 recreational fishers indicated they had used any type of bait in the previous 12 months. Those who used prawns or shrimp (3,396) were asked if they had purchased the prawns from a seafood retailer or a bait supplier. Those recreational fishers who reported using prawns purchased from a seafood retailer (822) were asked about the prawn (product) type, source (Australian origin or imported) and reasons for purchasing prawns from a seafood retailer rather than a bait supplier.

Use of prawns intended for human consumption as bait or berley for recreational fishing

For the 2002 and 2007 surveys ‘sold as seafood’ prawns were defined as prawns which were presented or sold as seafood, that is, intended for human consumption (Kewagama Research 2002, 2007). Conversely, ‘sold as bait’ prawns were defined as prawns which were presented or sold as bait (Kewagama Research 2002, 2007). The 2002 survey reported that only 6.8% of recreational fishers used prawns ‘sold as seafood’ as bait or berley (Kewagama Research 2002). However, by 2007, 7.9% of the ‘repeat fisher group’ were using prawns ‘sold as seafood’ as bait (Kewagama Research 2007).

Reports suggest that the use of prawns, intended for human consumption, as bait or berley by recreational fishers has increased well above that reported in the 2002 and 2007 surveys. In 2017, data from online surveys conducted by the Queensland Department of Agriculture and Fisheries found that 19% of fishers had used prawns bought from a supermarket as bait in the last year (Biosecurity Queensland 2017). A total of 768 fishers living in Queensland participated in this survey (Biosecurity Queensland 2017).

In 2017, the Kantar Public survey found that 23% of fishers reported using uncooked prawns purchased from a supermarket and 6% reported using left-over cooked prawns from a meal as fishing bait. A follow-up survey, of 750 recreational fishers living in Queensland, was conducted in 2019 and the results were not statistically different (Kantar Public 2019). During the department’s investigations following the WSD outbreak in South-East Queensland in 2016–17, 6.3% (9/144) of recreational fishers interviewed reported using raw prawns, intended for human consumption, as bait (Department of Agriculture and Water Resources 2017c). The 2019–20 Bait and berley survey reported that nationally, of the respondents who indicated they had used prawns as bait, 20.3% indicated that they had purchased bait from seafood retailers in the previous 12 months. By

jurisdiction, the highest proportion of prawns purchased from seafood retailers and used as bait was in the Northern Territory (32.4%), and the lowest was in Tasmania (4.1%) (Moore et al. 2022).

The Kantar Public survey (2017) also reported that 11% of fishers 'strongly agreed' that raw supermarket prawns were their preferred form of bait (Kantar Public 2017). In the 2019 follow-up survey, the group who 'strongly agreed' that raw supermarket prawns were their preferred bait was consistent with the 2017 responses (Kantar Public 2019).

Data from the 2007 survey indicated an increase in the amount of 'sold as seafood' prawns being used as bait compared to the volume used in 2002 for the same fishers. When data from the 'repeat fisher group' from 2002 was compared to their data from 2007, there was an increase of 18% (50.5 tonnes and 59.6 tonnes, respectively) in the volume of prawns purchased from seafood outlets and used as bait or berley in Australia. However, the apparent 9 tonnes increase should be treated with some caution given that the 95% confidence intervals for the 'repeat fisher group's' tonnage estimates were 29.8–89.4 tonnes (2007) and 12.2–88.8 tonnes (2002) (Kewagama Research 2002, 2007). The Kantar Public surveys did not report or estimate volumes of prawns used as fishing bait. The 2019–20 Bait and berley survey did not report or estimate total volumes of prawns used as fishing bait, but it did gather information about the individual usage ranges. Uncooked whole prawns were the most common prawn type purchased from a seafood retailer for use as bait (82%), with 24% reporting using >1 kg; and 58% reporting using <1 kg. This was consistent across New South Wales and Australian Capital Territory, Victoria, Queensland and Western Australia (Moore et al. 2022). It is assumed these prawns are Australian origin given the import conditions for uncooked, whole prawns in place at the time of the survey.

Data from the 2002 and 2007 surveys estimated that 1.7 tonnes and 6.3 tonnes (respectively) of prawns used by the 'repeat fisher group' as bait was potentially imported (Kewagama Research 2007).

During investigations into the WSD outbreak in South-East Queensland in 2016–17, the department became aware of instances of recreational fishers using imported prawns as fishing bait (Department of Agriculture and Water Resources 2017d). Two recreational fishers were located fishing upstream of a prawn farm with raw imported *P. vannamei* intended for human consumption. It was the third time they had fished in that river using prawns for human consumption. The remainder of the prawns were provided to department investigators who traced the import history of the prawns and determined they had been imported approximately 6 months prior and had tested negative (for WSSV and YHV1) at the time of import and were released for sale. Samples from the fisherman were sent for virus testing and tested positive for WSSV. The department undertook actions relating to non-compliance with import conditions concerning uncooked imported prawns (Department of Agriculture and Water Resources 2017d).

The Queensland Department of Agriculture and Fisheries online survey in 2017 looked at the behaviours of recreational fishers. When questioned about their awareness of the origin of prawns purchased from a supermarket for use as bait or berley, 6% of respondents were aware that they were imported prawns, 9% were aware they were Australian origin and imported prawns, 31% were not sure of the origin and the remainder were Australian origin prawns (54%) (Biosecurity Queensland 2017). The 2019–20 Bait and berley survey asked respondents that used prawns as bait sourced from a seafood retailer whether they had used Australian or imported prawns. Most

respondents (78%) said they either often used Australian-origin prawns or sometimes used Australian-origin prawns (45.8% and 32.7%, respectively) (Moore et al. 2022). Conversely, 33.5% of respondents who purchased prawns from a seafood retailer said they either often used imported prawns or sometimes used imported prawns (9.4% and 24.1%, respectively) (Moore et al. 2022). 46.8% stated they did not use imported prawns and 19.7% did not know if the prawns were imported (Moore et al. 2022).

Preferred form of prawns intended for human consumption but used as bait or berley for recreational fishing

The Prawn IRA 2009 considered that head and shell removal would reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. This assumption was based on evidence from the 2007 survey that recreational fishers preferred whole prawns (Kewagama Research 2007). There was no information since the 2007 survey about whether recreational fishers still prefer whole prawns for use as bait or berley. However, during investigations into the WSD outbreak in South-East Queensland in 2016–17, the department became aware of instances of recreational fishers using imported prawns as fishing bait (Department of Agriculture and Water Resources 2017d). Prawns imported at that time were required to have the head and shell removed. When considered with the increased reporting of the use of peeled prawns to bait hooks for recreational fishing in 2007 (17% up from 8% in 2002, noting small respondent numbers of 46 and 17 respectively) (Kewagama Research 2002, 2007), it indicates that recreational fishers use peeled prawns as bait or berley. It is likely that head and shell removal does not remove the attractiveness of these products for use as fishing bait or berley.

The 2019–20 Bait and berley survey reported that whole uncooked prawns were still the preferred form for prawns used as bait. However, of the respondents who purchased prawns for bait from a seafood retailer, 40% reported using uncooked but shelled prawns, with 34% reporting using less than 1 kg and 6% reporting using more than 1 kg. This demonstrates that this form of prawn is still attractive for use as bait but that whole uncooked prawns are the preferred form. Importantly, the 2019–20 Bait and berley survey found that cooked prawns, both shelled and not shelled were also used as bait. With 41% of respondents who purchased prawns as bait from a seafood retailer, indicating they had used cooked but not shelled prawns and 32% saying they had used cooked and shelled prawns for bait use. 24% of respondents who purchased prawns as bait from a seafood retailer reported using processed prawns (for example, skewers, marinated, breaded or battered, part of a dumpling, spring roll or other product, or butterflied), with 18% reporting using less than 1 kg in the 12 months prior to the survey, compared to 6% reporting using more than 1 kg. It is important to note that skewered, butterflied and marinated prawns are considered as uncooked prawns. Therefore, the usage of breaded, battered and crumbed and dumpling-type products as bait may be less than what is reported in these usage patterns as the skewered *et cetera* products likely make up a large proportion of the use in this category.

Why recreational fishers use prawns intended for human consumption as bait or berley for recreational fishing

There are several factors which impact why recreational fishers use prawns, intended for human consumption, as fishing bait or berley. The 2002 survey identified the key reasons to be (in order of importance): freshness/quality (46%), convenience (23%) and price (16%) (Kewagama Research 2002). The 2007 survey identified (in order of importance) convenience/access (47%), freshness/quality (34%) and price (15%) as the main reasons for purchasing ‘sold as seafood’ prawns

(Kewagama Research 2007). There was a significant increase between 2002 and 2007 in the reporting of convenience/access as the key factor for using prawns 'sold as seafood' (Kewagama Research 2007). There is also evidence that the low retail price of 'sold as seafood' prawns and increased availability has meant they are purchased more frequently by recreational fishers for use as bait (Kantar Public 2017; Kewagama Research 2002, 2007).

The Kantar Public survey in 2017 identified that convenience (71%) and price (56%) were the main drivers for using 'sold as seafood' prawns as bait (Kantar Public 2017). The Kantar Public follow up-survey in 2019 identified the same pattern of behaviour, with no statistical difference in the responses (Kantar Public 2019).

In the 2017 online surveys conducted by the Queensland Department of Agriculture and Fisheries, it was found that cost (34%), availability (convenience) (28%) and quality (21%) were the main reasons that fishers used prawns purchased from a supermarket as bait (Biosecurity Queensland 2017).

The 2019–20 Bait and berley survey asked respondents to select out of 10 categories their reason for obtaining prawns from a seafood retailer than from a bait supplier. The primary reasons reported nationally for using prawns from a seafood retailer rather than prawns from a bait supplier were, better quality than bait prawns (36%), freshness (34%) and lower cost (26%) (Moore et al. 2022). The surveys discussed in this report show a steady increase since 2002, and more recently a relatively stable driver, of 'convenience and freshness or quality' as the primary reasons why prawns 'sold as seafood' are used as bait or berley.

In response to the biosecurity risks associated with using imported prawns as recreational fishing bait, recent public awareness campaigns have been conducted at state and territory government, industry and community levels. The goal of these awareness campaigns has been to educate fishers about the disease risks associated with using 'sold as seafood' prawns as fishing bait. The effectiveness of these campaigns are questionable given apparent knowledge rates of recreational fishers with respect to these issues.

New South Wales Department of Primary Industries (NSW DPI) Fisheries Compliance reported that they continue to observe recreational fishers using prawns intended for human consumption for bait despite extensive education and awareness campaigns highlighting the risks associated with this activity (NSW Department of Primary Industries 2018). NSW DPI also report it is not practical, possible or an efficient use of resources to ensure that human consumption prawns are not used as bait (NSW Department of Primary Industries 2018).

When asked, fishers in Queensland had limited unprompted awareness of relevant and correct information relating to WSD (Kantar Public 2017). This was despite the survey occurring during the WSD outbreak and in a period of active communication and education campaigns about the issue. Whilst 77% of fishers surveyed said they had heard of WSD, only 51% knew of the recommendations and restrictions that were in place to help prevent its spread (Kantar Public 2017). Results in 2019 were statistically consistent with 2017 (Kantar Public 2019). In 2017 and 2019, of those fishers who were aware of the recommendations and restrictions, around one in ten were still not following them (Kantar Public 2017, 2019). For those fishers who were unaware of the WSD recommendations and restrictions, approximately four in five demonstrated the incorrect behaviour (Kantar Public 2017).

The Kantar Public survey (2017) reported that there was confusion from recreational fishers about the use of prawns intended for human consumption as bait, with the perception being that prawns sold in Australia must be safe (Kantar Public 2017). They did not understand how or why a product could be safe for human consumption, but not safe for consumption by aquatic animals. Further, the Kantar Public survey reported beliefs from a fisher(s) that (Kantar Public 2017):

> If you're going to allow prawns into Australia and sold in the shop it is going to be used. It doesn't matter if you put signs up or whatever… So you don't let the product in Australia. You don't say 'we'll let it into Australia, but people won't use it for bait.' They will use it for bait. It is as simple as that.

The department views this as likely a widespread belief given the driver for purchasing 'sold as seafood prawns' is convenience, freshness/quality and price.

In the 2019–20 Bait and berley survey, all respondents, regardless of whether they used prawns as bait or not, were asked if they had seen advice regarding the use of imported seafood prawns as bait. Nationally, 47% of respondents who answered this question said that they had seen advice on not using imported prawns as bait (Moore et al. 2022). The jurisdiction with the highest proportion of respondents who were aware of this advice was Queensland (68.2%), likely reflecting the education and awareness programs in that region at the time (Moore et al. 2022).

Jurisdictional controls over bait and berley use by recreational fishers

Each Australian state and territory has its own legislation related to fisheries, and requirements vary across jurisdictions. Aside from in the Northern Territory, the jurisdictions do not have specific legislation to prevent the use of imported seafood, intended for human consumption, as bait or berley. Some states and territories have legislation that could apply in cases of deliberate introduction of exotic pests or diseases into the aquatic environment. However, this legislation is not easily or readily enforceable. Appendix G provides a summary of relevant legislation for each state and territory.

Following the WSD outbreak, the Queensland Government implemented fishing restrictions around all prawn farms in the Logan River region. Whilst primarily intended to prevent further outbreaks of WSD, it may reduce the risk associated with introduction of potentially infected imported prawns into the environment close to prawn farms. These measures remain in place at the time this report was prepared.

2) *Use of imported prawns as feed for crustacean broodstock and crustaceans in research facilities and public aquaria*

Uncooked prawns were known to form a significant component of broodstock conditioning diets (Chimsung 2014; Coman et al. 2007; El-Bermawi 2010; Wouters et al. 2001). In the past it was widespread practice to condition broodstock using uncooked, frozen seafood, with whole prawns being the preferred option from a nutritional perspective (Chimsung 2014; Coman et al. 2007; El-Bermawi 2010; Wouters et al. 2001). This is primarily because prawn head meal contains growth promoting factors (Sudaryono et al. 1995; Williams et al. 2005). Nowadays marine invertebrate meals such as squid liver meal and prawn meal, as well as fresh/frozen seafood such as clams, mussels, snails, and polychaetes are regularly used by the prawn industry for growth and propagation (Simon et al. 2019). Whilst being the preferred diet, prawns and other crustaceans are generally now excluded from prawn maturation regimes due to the risk of disease transmission (Australian Prawn

Farmers Association 2021; Chimsung 2014; Department of Agriculture and Fisheries 2021; El-Bermawi 2010; Wouters et al. 2001). Practices for maturation of broodstock prawns now include the use of a mixture of pelleted feeds and fresh feeds that mainly include polychaetes and molluscs (squid and mussel) (Braga et al. 2010; Chimsung 2014; El-Bermawi 2010; Emerenciano et al. 2013; Mandario 2018; Wouters et al. 2001).

The Prawn IRA 2009 considered that feeding large adult prawns (held in farm grow-out ponds until maturation) with whole imported prawns represented a significant and direct pathway for the potential exposure of farmed crustaceans. It was also considered that whole imported prawns may be used to feed crustacean broodstock in hatcheries. In 2000, broodstock in a Northern Territory crustacean hatchery were fed imported prawns and developed white spot disease. In that instance, the prawns, imported for human consumption, were of poor quality (based on smell), and were subsequently repackaged, unlabelled and diverted into the bait market where the prawns were purchased and used to feed hatchery broodstock. The broodstock from the 2000 Northern Territory incident were destroyed once the source of the feed prawns was realised. It is acknowledged that the incident in Darwin occurred some time ago.

The department has been advised by the jurisdictions and industry that feeding of broodstock kept on farms or in hatcheries with whole uncooked prawns no longer occurs in Australia (Australian Prawn Farmers Association 2021; Department of Agriculture and Fisheries 2021). Although, the department is aware of seafood (non-crustacean based) imported for human consumption, being used as feed for hatchery animals in Australia in 2017.

If imported prawns were used as feed in farms or hatcheries it would be a direct and potentially significant exposure pathway with a high likelihood of completion, and whilst based on the current practices in the industry, it is unlikely this would occur, it nevertheless is still considered a major exposure pathway. However, the department has reduced the exposure likelihoods for farmed crustaceans in light of the information provided by industry and the jurisdictions.

The Prawn IRA 2009 also identified that conditioning and feeding of crustaceans is not limited to the hatchery or farm setting. Fresh seafood is a primary dietary component for feed used in research facilities, teaching institutions and public aquaria throughout Australia (Biosecurity Australia 2009). States and territories do not have legislation (see Appendix G) in place to regulate this behaviour and the department is of the view this likely still occurs. Although the volume of imported prawns used to feed crustaceans in research and public aquaria would be very small, it represents a direct and potentially significant exposure pathway (with a high likelihood of completion) by which crustaceans in research facilities and public aquaria (part of the hatchery exposure group) could be exposed to a hazard. Crustaceans kept in hatcheries, research institutions and public aquaria were considered overall unlikely to be deliberately or inadvertently exposed to imported prawns used as bait or berley. This is due to the more stringent biosecurity and physical containment implemented in these facilities. However, the deliberate use of imported prawns as feed for crustaceans in research institutions and public aquaria could occur because this is an unmonitored, unregulated exposure pathway.

Imported uncooked prawns used as a fresh feed represents a high-risk exposure pathway, as any hazards present would be subject only to the minimal inactivation associated with freezing and thawing of prawns.

Amount of infectious hazard in imported prawns (or associated wastes) at point of exposure

The amount of infectious hazard present will depend on numerous factors including the infectious dose and pathogenic agent stability.

Infectious dose

For most hazards considered in this risk review, data are not available describing a 'true minimum infectious dose'. This is because there are no continuous crustacean cell lines, for titration of viruses, which are required to ascertain a 'true minimum infectious dose.' However, due to advances in qPCR techniques, there are some studies describing infectious doses of the hazards. For example, in an experimental study, *Penaeus monodon* were challenged by intramuscular injection with 0.1ml of WSSV stock at 2.62×10^6 genome copies/µL that was sufficient to result in moribund prawns within 72 hours post-infection (Gomathi, Otta & Shekhar 2015). Since prawns at the onset of mortality are reported to have WSSV loads in the order of 10^9–10^{10} copies/g of tissue (Oidtmann & Stentiford 2011), one WSSV-infected prawn tail (approximately 12g if a harvested prawn weighed 20g) could contain 458–4580 WSSV infectious doses, which would be more than sufficient to cause infection if it were used for bait or berley and consumed by a susceptible species. It is noted challenge by intramuscular injection is not a natural means of exposure and does not mimic natural exposure routes.

Ability of pathogenic agent to remain infectious at point of exposure

The ability of pathogenic agents present in prawns (or associated wastes) to persist and remain infectious at the point of exposure to a susceptible crustacean depends primarily on the stability of the pathogenic agent through normal processing, transport and storage. For example, freezing and thawing would decrease the amount of some infectious pathogenic agents such as Vp AHPND (OIE 2021b; Tran et al. 2013a). Other hazards, such as WSSV, can persist and maintain infectivity in frozen prawns for extended periods (Durand & Lightner 2002) and would therefore be expected to be infectious at the time of exposure.

The ability of a pathogenic agent to remain infectious when in water for extended periods is also an important consideration. For example, WSSV can remain infectious in seawater for up to 120 days at 15°C (Momoyama et al. 1998) and for 3–4 days in ponds (Nakano et al. 1998).

Prawns that are used as feed for crustaceans or as bait or berley represent a potentially high-risk exposure pathway because any hazards present would only be subject to minimal inactivation associated with freezing and thawing. Freezing and thawing may affect the virions of some pathogenic agents such as YHV (Wongteerasupaya et al. 1995a). However, freeze-thaw cycles do not affect others. For example, TSV reportedly survives multiple freeze-thaw cycles in prawn tissues (Hasson et al. 1995).

Contact between susceptible host animals and imported prawns (or associated wastes)

In Australia, the main aquaculture species are *P. monodon* and *Penaeus merguiensis* (Australian Prawn Farmers Association 2019; State of Queensland 2021). The main target species for fisheries includes *P. merguiensis, Penaeus indicus* and *P. monodon* (Mobsby & Curtotti 2020; Steven, Dylewski & Curtotti 2021) (refer Table 3 for complete list).

Farmed crustaceans are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported prawns (or associated waste) introduced to farmed and hatchery crustacean exposure groups would encounter, and likely be consumed by them.

The probability of wild crustaceans encountering imported prawns (or associated wastes) depends on several factors. These factors include the volume of product released into the natural environment, the dispersal and dilution of that material, the presence and concentration of susceptible crustaceans in the area, and the proportion of material that might be consumed by other non-susceptible species in the vicinity.

Wild susceptible crustaceans would be less abundant than crustaceans in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. In the wild, crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley.

Wild prawns (or susceptible crustaceans) are considered moderately likely to encounter prawn material introduced into their environment. However, because of greater competition from other aquatic animals (especially fish, crabs and other crustaceans), only a small proportion of such material may end up being ingested by wild prawns (AQIS 1999). Wild finfish are highly likely to access any prawn material entering their environment and are likely to ingest a moderate to high proportion of any such material. Wild crustaceans would be expected to ingest only a small proportion of prawn material entering their environment. However, the amount ingested may still be sufficient to cause disease dependent upon the hazard's infectious dose and the range of susceptible host species. For hazards with a wide host range such as WSSV, the likelihood of wild susceptible crustaceans encountering that hazard is relatively high in comparison to those hazards with a smaller host range, such as IMNV. Other (non-crab and non-prawn) wild crustaceans may also encounter prawn material in estuarine environments but are unlikely to be exposed to prawn material in open ocean environments.

Consequence assessment

According to the WOAH Code, a consequence assessment should describe the potential consequences of a given exposure and estimate the probability of them occurring (WOAH 2022c).

For this risk review, the steps were taken to assess the 'likely consequences' associated with each hazard were:

- Identifying a likely outbreak scenario that may occur from host exposure to the hazard.
- Estimating the likelihood of that outbreak scenario occurring to obtain a 'partial likelihood of establishment and spread' for the outbreak scenario.
- Determining the level and magnitude of adverse impacts (economic, environmental and social) resulting from the outbreak scenario.
- Combining the 'partial likelihood of establishment and spread' with the corresponding estimation of impacts to obtain the 'likely consequences' for each exposure group.

Identification of the outbreak scenario

The Prawn IRA 2009 considered two most likely outbreak scenarios:

- Outbreak scenario 1: the agent establishes and spreads to wild and farmed populations of susceptible species in Australia—it is assumed that if an agent were to establish in a local population it would eventually spread to its natural geographical limits.
- Outbreak scenario 2: the agent does not establish—an index case may occur and infection may spread to co-habiting animals, but the agent does not persist sufficiently long to be detected.

It was noted in the Prawn IRA 2009 that eradication of an aquatic animal disease is not generally feasible and that in the aquatic environment, if a disease does establish in a population following exposure, it is generally not possible to prevent its spread by natural means. The Prawn IRA 2009 further stated that based on the effectiveness of control and eradication programs for aquatic animal diseases, and the speed at which authorities would be able to detect outbreaks, control and eradication are generally not viable. Such an approach was considered suited to the unique situation in aquatic environments where the number of meaningful outbreak and response scenarios is generally limited, compared to terrestrial environments. In the terrestrial situation, there may be a wider range of likely outbreak scenarios depending on such factors as livestock management practices, the epidemiology of the pathogenic agent, and established control and eradication programs.

Several possible outbreak scenarios may follow exposure of a susceptible population to a hazard. These scenarios represent a continuum ranging from no spread, to establishment and spread of the disease to its natural geographic limits. For this risk review, one outbreak scenario was assessed because it has the most potential to occur with significant consequences:

> The hazard establishes in the directly exposed population and spreads to wild and farmed populations, is not eradicated, becomes endemic in Australia and eventually spreads to its natural geographical limits.

This is consistent with other risk reviews conducted by the department whereby only one outbreak scenario is assessed (for example, *Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses—final import risk analysis report* (Department of Agriculture 2014b)). Additionally, the consideration of an outbreak scenario where the impacts are *negligible* (such as with the case of outbreak scenario 2 from the Prawn IRA 2009) does not change the overall risk outcome.

Partial likelihood of establishment and spread associated with the outbreak scenario

The factors considered relevant when estimating the 'partial likelihood of establishment and spread' (PLES) were:

- infectious dose
- mechanisms of spread and transmission
- susceptibility of Australian species to infection
- predation of infected tissues and animals.

Infectious dose

The likelihood that a hazard will establish and spread, is affected by how easily the hazard can be transmitted from an index case to other susceptible animals. This is influenced by the dose of the

hazard necessary to cause infection and the likelihood that susceptible animals would be exposed to that dose. For most hazards considered in this risk review, data are not available describing a 'true minimum infectious dose' (refer Infectious dose for further details). The environmental conditions at the time of infection, the density of susceptible animals and the health and immunological status of the recipient host animal, would also have to be considered. Transmission from an index case(s) to other susceptible species may occur through ingestion of infected animals or exposure to free hazard (including in waste such as faeces) in the water column.

Transmission of tissue bound pathogenic agents is more likely to occur orally by susceptible animals feeding on infected material. Whether a susceptible species would receive an infectious dose by feeding on an infected animal is crucial to whether a hazard can establish and spread.

The likelihood of establishment and spread will also be impacted by the amount of each hazard present in the environment (through for example, shedding by infected animals), especially in the case of waterborne transmission. Hazards, which have a low minimum infective dose, will be more capable of spreading through the water even in cases of large, dispersed areas of animals. Those hazards, which have higher minimum infectious doses, will be less capable of establishing and spreading.

The effect of dilution is also an important consideration when determining whether a host animal will be exposed to an infectious dose of a hazard, and therefore the likelihood of whether a hazard will establish (and ultimately spread) within a population. For example, prawn farm effluent in Australia might be treated through settlement, dilution and screening before it is released into natural waters. This could reduce the amount of pathogenic agent (or dose) encountered by a susceptible animal, as well as reducing the likelihood of spread to wild crustaceans or other farms. This settlement process will also reduce the likelihood of escapees, which reduces the likelihood of spread to other exposure groups. It may be less likely that large numbers of dead or live prawns will escape prawn farms under the usual circumstances. However, the effect would be less in cases where water effluents are not treated regularly, and significant quantities of effluent water and potentially infected animals are released to the environment before a biosecurity response is enacted in response to the identification of a hazard. Also, if there was an accidental release of a large number of animals from a farm and they were infected with a hazard, the effect of dilution under this circumstance would be less, due to the ability of potentially susceptible animals (that is wild crustaceans) to detect and capture food material (or otherwise encounter an infected prawn), notwithstanding competition from non-susceptible species.

Mechanisms of spread and transmission

The dispersal of pathogenic agents can occur via several pathways. In the wild pathogenic agents are typically dispersed by the movement of live hosts, including during natural migration (Biosecurity Australia 2009). In farmed prawns, movement of infected broodstock to hatcheries and infected larvae from hatcheries to grow-out ponds has facilitated national and international spread of pathogenic agents. For example, the introduction and spread of TSV throughout the Americas has been attributed to the movement of infected broodstock and postlarvae (Brock 1995; Lightner 1995).

For most pathogenic agents of prawns with a direct lifecycle, infection usually occurs as a result of the introduction of a live, infected host into a naive (and susceptible) population, either from waterborne transmission through shedding of the pathogenic agent into the water or orally, via

ingestion of infected host tissues. Transmission from broodstock to progeny has been reported for some pathogenic agents and may occur via infection of the eggs, via contamination of the external surface of the egg, or via release of the pathogenic agent during spawning which is subsequently ingested by the larval stages (for example, WSSV) (Chang, Chen & Wang 1998b). Some pathogenic agents may cause subclinical infection, so apparently normal, infected prawns may still be a source of infection. Vectors and hosts may play a role in the mechanical spread of pathogenic agents. For example, seagulls (*Larus atricilla*) and the water boatman (*Trichocorixa reticulata*) have been shown to serve as mechanical vectors for TSV (Garza et al. 1997; Vanpatten, Nunan & Lightner 2004). The greater the population density of host animals susceptible to disease, the more readily disease may be transmitted, resulting in higher morbidity and increased likelihood of pathogenic agent establishment.

In addition to the density of susceptible species, other factors that affect the susceptibility of the host to infection (for example, life-cycle stage, the health and immunological status for the host, environmental conditions, and intercurrent stress) may also affect transmission. Evidence of experimental transmission that mimics natural pathways is considered when specific information on natural transmission of the pathogen is unavailable or unknown.

Prawn farms in Australia generally pump seawater into pond systems from coastline areas and river inlets. Many Australian prawn farmers practise minimal water exchange policies in the interests of improving environmental management practices and sustainable aquaculture. The dispersed nature of the prawn aquaculture industry in Australia, and the trend of reducing water exchange rates, may help to prevent rapid spread of prawn hazards between farms and spread from farms to wild crustaceans outside of directly affected regions or zones. This was demonstrated in the Logan River WSD outbreak whereby farms outside of the Logan River were not infected and there were no WSSV positive test results in wild crustaceans outside of the movement regulated area. However, the spread of a hazard between farms that are not geographically isolated and that have a common water supply is likely (as was the case with prawn farms on the Logan River).

The Prawn IRA 2009 considered that the spread of disease between farms might be exacerbated by the limited extent of structured surveillance and disease control policies in some states or territories (or jurisdictions), as well as the generally limited biosecurity measures applied to the translocation of locally caught broodstock and their postlarvae between farms. Since that time there have been improvements to inter-jurisdictional aquaculture oversight and the introduction of health management practices for translocation of broodstock and postlarvae. For example, Queensland have put in place the Health protocol for the movement of live prawns which applies to all prawns caught for the purposes of being used as broodstock in the prawn farming sector. This protocol also manages the movement of live prawns into and within Queensland. Movement of broodstock and postlarvae into New South Wales for stocking into New South Wales farms is managed through a Health protocol for translocation of prawn post-larvae into NSW for stocking into NSW prawn farms for the 2019 season. New South Wales does not have any restrictions on movements of live prawns within the state, where prawn farming operations are concentrated in the northern end of the state. It is however noted that these protocols will not detect disease incursions which may occur through pathways other than translocation. For example, identification of disease on a farm or hatchery, which has occurred through a pathway other than translocation of broodstock or postlarvae, is

reliant upon the farms identifying and notifying jurisdictions of a possible disease (both endemic and exotic) event.

Spread from wild crustaceans to farmed crustaceans is a potential pathway for establishment and spread. The department noted during its investigations into the WSD outbreak in farms on the Logan River in South-East Queensland in 2016–17 that biosecurity measures on some farms were lacking at that time. None of the farms had crab-proof fences, which enabled the movement of crabs in and out of the river, between ponds and between farms. Similarly, some of the farms did not have in place measures to prevent bird predation. Some farms also lacked effective water filtration methods. Consequently, wild prawns and crabs could enter into ponds and grew there alongside farmed prawns, or were allowed to grow in inlet channels (Department of Agriculture and Water Resources 2017c). Crab-proof fencing and inlet channel filters could help in reducing the presence of wild prawns and crabs entering farms. The presence of wild crustaceans in inlet channels increases the likelihood of movement of hazards onto the farm. This is because the amount of hazard entering the farm may be high in circumstances of large population of diseased wild crustaceans living in close proximity to inlet channels. Some hazards are able to remain viable in the water column for long periods of time (for example, WSSV can remain infectious in seawater for up to 120 days at 15°C (Momoyama et al. 1998) and for 3–4 days in ponds (Nakano et al. 1998). These gaps in on–farm biosecurity increase the likelihood of indirect exposure of farmed crustaceans to hazards (through exposure to infected wild crustaceans or exposure to free hazard in the water). The department understands that those farms who have resumed production in the WSSV movement regulated area have improved their biosecurity systems. However, this is not believed to be the case for some prawn farms outside of the Logan River area (Wesche, Beattie & Crook 2019).

Susceptibility of Australian species to infection

Most reports of prawn pathogens are from *P. vannamei* which is not present in Australia, however many of the prawn pathogens also affect species that are commercially important in Australia.

Some of the hazards in this risk review are host-specific and infect only one or several prawn species from the same genus. For example, IMNV only infects some *Penaeus* species (OIE 2021g). Other hazards have a much wider host range and can infect multiple genera, other groups of crustaceans and even other arthropod groups. For example, WSSV can infect prawns, crabs and crayfish (OIE 2021k) and CMNV is known to also infect finfish (Wang et al. 2018; Zhang et al. 2018). Hazards that have a very wide host range have a higher likelihood of establishing in Australia.

Australian prawn populations are likely to be at least as susceptible to infection with a pathogenic agent as the same species found in other regions. In some cases, the Australian populations may be more susceptible as they will not have prior exposure or host adaptation to the hazards. On the other hand, environmental and husbandry conditions that might favour the expression of disease in prawn populations in other regions may not be present in Australia. The effects of some hazards (for example, WSSV and Vp AHPND) in prawn aquaculture throughout Asia are considered to have been exacerbated by environmental pollution and other stressors (Flegel & Sriurairatana 1994; Thitamadee et al. 2016). Season, or time of year, can affect the likelihood of establishment and spread. For example, outbreaks of WSSV occur more frequently in the monsoon season due to stressors such as fluctuations in salinity, water temperature and pH (Karunasagar, Otta & Karunasagar 1997; Korkut, Noonin & Söderhäll 2018; Peinado-Guevara & Lopez-Meyer 2006).

Predation of infected tissues and animals

A review of the scientific literature for the Prawn IRA 2009 found that natural mortality of crustaceans, and in particular wild prawn populations (including due to predation), is high (Biosecurity Australia 2009). This conclusion is still valid.

Prawns are important components of the lower trophic levels of the natural food chain in the wild and are subject to high predation pressure (Salini, Blaber & Brewer 1990). Predation is a major contributor to the high mortality of postlarvae, juvenile, sub-adult and adult prawns in the wild, with predation being the greatest cause of mortality in some prawn species (Minello, Zimmerman & Martinez 1989; Salini, Blaber & Brewer 1990). The risk of predation could increase many folds if infection resulted in some level of morbidity. Equally, the infected animals might die of other causes and be removed by scavenging finfish, crabs or other animals. In turn, non-prawn scavenging crustaceans, particularly brachyurans (crabs) in marine environments, are also a major prey population for fish (Salini, Blaber & Brewer 1994).

Predation of commercially important penaeid prawns by fish predators is influenced by environmental factors and habitat types that have an effect on the type of predator and prey species present (Salini, Blaber & Brewer 1990, 1994; Salini, Brewer & Blaber 1998). The mangrove habitats associated with many prawn farming areas in Australia are considered ideal in the context of providing protection of escaped farm prawns from predatory finfish, thereby providing a pathway for the exposure of wild crustaceans to hazards associated with farmed crustaceans (Nagelkerken et al. 2008). Despite the apparent environmental protection, high levels of predation of prawns have still been reported in nursery areas of the Norman River estuary in Queensland, which supports large populations of *P. merguiensis* (Salini, Brewer & Blaber 1998). If a limited number of index cases of infection did result from the exposure of wild prawns to a hazard, the infected animals are most likely to be consumed by predatory finfish (Flegel 2020), thereby limiting the likelihood of the hazard spreading more widely within the population (Biosecurity Australia 2009).

The likelihood of the establishment of a hazard in wild crustacean populations would be reduced by predation of prawns and crustaceans by non-susceptible species. However, if the density of susceptible crustaceans in the wild is high, relative to fish and other predators, the probability of disease spreading in a wild crustacean population would be greater. In this context, there is no predator density associated with farmed and hatchery crustaceans.

The Prawn IRA 2009 considered that the escape *en masse* of infected farmed prawns into the wild would pose a greater risk to wild prawn populations than exposure of wild prawns to recreational fishing bait (Biosecurity Australia 2009). This scenario includes the continuous escape of small numbers over an extended period. The department considers this conclusion remains valid since disease spread from prawn farms to wild populations has been reported previously (Biosecurity Australia 2009; Chang et al. 2004; Lightner et al. 1997b; Mijangos-Alquisires et al. 2006; Withyachumnarnkul et al. 2003). For example, gill associated virus (GAV) is considered to have spread into the Joseph Bonaparte Gulf through escapees from Northern Territory prawn farms (Biosecurity Australia 2009). In another example, wild *P. vannamei* from the Gulf of California inhabiting a coastal zone with high prawn aquaculture activity were shown to be infected with WSSV, having previously tested WSSV-negative (Mijangos-Alquisires et al. 2006). However, it is highlighted that escape of infected farmed prawns is a less likely scenario than the exposure of wild crustaceans to imported prawns through bait and berley use.

Adverse (economic, environmental and social) impacts

The potential adverse impacts of establishment and spread may be direct or indirect. They were evaluated against seven (two direct and five indirect) impact criteria.

Impacts may occur over an extended period and consideration of them is not limited to what might occur during one year, but covers a period as long as impacts are discernible.

The direct and indirect impacts described collectively cover the economic, environmental and social impacts of an outbreak—the so-called 'triple bottom line'. In assessing direct and indirect impacts, impacts were not considered more than once. In particular, the direct impacts of a disease on a native species were assessed under the criterion describing the 'the environment (native animals/plants, and non-living environment)'. The indirect or 'flow-on' effects on the environment were assessed under the last two indirect criteria.

When assessing impacts, the frame of reference was the impact of each hazard on the Australian community, rather than on the directly affected parties. A related consideration is the persistence of an effect. If the effect is prolonged, as would be the case if the hazard were expected to persist for several production cycles, or if restocking following eradication programs was expected to take several generations, the consequences were considered greater. If an effect is not expected to be prolonged, then consequences are considered less likely to be serious.

Direct impacts

Direct impacts are those on:

- the life or health (including production effects) of domestic or feral animals
- the environment, including life and health of native wild animals and direct effects on the non-living environment.

Animal health (production losses in aquaculture and commercial fisheries)

The biological effect of disease depends on the interaction of the environment, hazard and host. The nature of this interaction reflects factors specific to the hazard (such as virulence and infectivity), the host (such as susceptibility, immune competence and population density), and the environment (such as quality and availability of habitat for susceptible hosts). The one-pathogen-one disease paradigm is shifting however, and it is now hypothesised that hazards do not operate in isolation but rather as a part of a microbial consortium that is present within the host—termed the 'pathobiome' (Bass et al. 2019). In this scenario, the biological effect of disease will depend on the interactions between multiple organisms, the host and the environment.

Normally the biological effect of disease is evaluated in terms of morbidity and mortality. Evaluation of morbidity includes reduced production, which is described by parameters such as food conversion efficiency and fecundity of a population under study. Diseases that reduce the efficiency of production without causing large increases in mortality are more likely to be significant in farmed prawns than wild-caught prawns.

In farmed prawns, 'normal' or baseline values for production and mortality are often highly variable, reflecting husbandry practices, stocking rates and stress. The generally higher prevalence of disease and the frequent emergence of new disease problems in farmed prawns supports the view that farmed prawns are subject to more environmental stresses and higher disease transmission rates

due to high population density compared to wild prawn populations. It also reflects closer monitoring of farmed prawns than wild prawn populations.

The impact of an exotic pathogenic agent in Australia may not be the same as that seen overseas. This could especially be the case with new viruses where the impact may depend on the overall effect of the new virus acting in combination with the suite of viruses already endemic in Australian host populations, the underlying resistance or susceptibility of Australian crustaceans to that virus and environmental conditions.

The underlying 'baseline' or 'normal' rate of mortality in wild populations can be estimated from data collected in studies of population density, age/size structure and catch rates. Population fluctuations can be linked quite closely to other factors, such as fishing pressure, using these sorts of data. However, only major epidemics involving significant mortalities or grossly visible clinical signs are likely to be detected in wild host populations.

In the wild, disease is a component of natural mortality that is difficult or impossible to estimate, except in general terms. Prawn populations may fluctuate by orders of magnitude for a variety of reasons, including environmental changes. In addition, stock assessment of wild fisheries is an imprecise science because population estimates of prawn stocks have high coefficients of variation. As a result, a disease event may kill a large proportion of the population without detection.

The ability to accurately assess impact of disease on wild crustacean fisheries in terms of production losses is far more challenging than similar assessments of farmed crustacean stocks (Stentiford 2012). Impacts of new diseases in wild populations of crustaceans are likely to go unnoticed in countries without proper baseline ecological data, and baseline surveys are critical but often lacking (Shields 2012). In addition, fisheries suffer from both direct losses (such as mortalities) and also indirect losses (such as stunting, castration, and increased risk of predation) due to diseases. Indirect losses can be significant but are often overlooked by the fishing industry because their primary focus is on recruits to the fishery and not on the affected juvenile pre-recruits (Behringer 2012; Shields 2012; Stentiford 2012).

Perhaps the best known epidemic of wild crustaceans is crayfish plague, caused by the oomycota *Aphanomyces astaci*, which has eliminated native freshwater crayfish from many river systems in Europe (FAO 2007; OIE 2021e). In marine environments, mass mortalities of krill by parasitic ciliates of the genus *Collinia* have been reported (Gomez-Gutierrez et al. 2003; Gómez-Gutiérrez et al. 2010). Another example is WSSV, which can infect a wide range of aquatic decapod crustaceans, including marine, brackish and freshwater prawns, crabs, crayfish and lobsters (OIE 2021k). WSSV was detected at a 2.8% prevalence in wild Atlantic prawn populations (*P. setiferus* and *P. aztecus*) of the south-east coast of the United States of America (Chapman et al. 2004) and was found in prawn samples from 3 out of 6 sites in the Philippines where wild *P. monodon* are caught (Orosco & Lluisma 2017). Although pathogenic agents have been detected in wild prawn samples, there is limited data relating to disease occurrences in wild populations of prawns that have led to a decline in fishery catches. Decapod penstylhamaparvovirus 1 (formerly known as infectious hypodermal and haematopoietic necrosis virus (IHHNV)) has been reported to contribute to the decline in the fishery for *P. stylirostris* in the early 1990s in the Gulf of California (Morales-Covarrubias et al. 1999; Pantoja, Lightner & Holtschmit 1999; Robles-Sikisaka et al. 2010; Tang & Lightner 2001). In addition, the parasite *Epipenaeon ingens*, has been reported to cause concerns about regeneration of stock in

catches of *P. semisulcatus* and *P. esculentus* from Northern Australia (Owens 1993b; Owens & Glazebrook 1985).

There is evidence that farmed prawn populations may rapidly develop tolerance or resistance to pathogenic agents that initially cause very serious disease. Better management of infected populations may also provide improved outcomes. Although this may be the case, relatively minor stress events may predispose latently infected prawns to clinical disease.

Initially, yellow head disease and later, WSD, were associated with widespread epidemics in prawn aquaculture in South-East Asia in the early to mid-1990s. In the latter 1990s, techniques to manage serious diseases in prawn aquaculture in the region combined with improved diagnostic techniques lessened the impact of disease. For example, by the end of the 1990s, prawn aquaculture production for Thailand was approaching pre-WSD levels (Flegel 1997b). The epidemiology of WSSV in severely affected regions was also altered. Flegel (1997b) noted that following the WSSV epizootic in Thailand, the prawns appeared to rapidly develop a kind of tolerance or resistance to the virus within a period of 1.5 years of it first causing high mortalities. Consequently, the proportion of aquaculture ponds now emergency harvested would be lower than at the height of the epidemics.

In some cases, the immune regulation of this putative tolerance developed by prawns has been linked to DNA markers. For example, a 71 bp microsatellite DNA marker was reported to be significantly present in WSSV-susceptible *P. monodon* and a WSSV challenge experiment showed that when this marker was present there was 1.21×10^3 fold higher WSSV viral load (Dutta et al. 2013; Mukherjee & Mandal 2009). In the case of TSV, resistance has been linked to both single nucleotide polymorphisms of heat shock protein 70 and multiple alleles in the M1 microsatellite marker (White et al. 2002; Xu et al. 2003; Zeng et al. 2008).

Similar mechanisms for the development of tolerance by farmed prawns to newly recognised pathogenic agents may occur in wild prawns or crustaceans. In addition, predation of clinically diseased prawns may limit spread of pathogenic agents in wild populations and favour the selection of highly tolerant or resistant strains of prawns.

The consequences of establishment of an exotic disease in Australian prawn aquaculture is assessed in relation to characteristics of the local industry (refer section 1.3 Australia's prawn industry for details about the industry in Australia).

This risk review takes the same approach as the Prawn IRA 2009 by assuming that farmed and wild prawns (including native species) in Australia would be at least as susceptible to infection as prawns of the same species, reported as susceptible under similar conditions in other countries. In the case of hazards shown by overseas experience to be highly pathogenic (for example, WSSV and YHV1), it has been assumed that, where susceptible species exist in Australia, rates of morbidity and mortality would be comparable to those reported overseas, unless there is evidence to the contrary.

The environment (native animals/plants, and non-living environment)

The establishment of a new disease could affect the survival of native species not farmed or otherwise commercially exploited.

To determine the likely effect of hazards on Australian native species, the department considered whether the hazards could infect a wide range of species or families, including any that are related to

Australian native species. In the case of hazards that infect a narrow or specific range of hosts that are unrelated to Australian species, it was assumed that effects on native species would be minimal. However, for hazards that have a wide or non-specific host range (including species that are related or similar to Australian species) it was assumed that native species would be susceptible to infection and that the consequences would be at least as severe as those reported overseas.

Indirect impacts
Indirect impacts are those on:

- new or modified eradication, control, surveillance or monitoring and compensation strategies or programs
- domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries
- international trade, including loss of markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand
- indirect effects on the natural environment, including biodiversity, endangered species, and the integrity of ecosystems
- indirect effects on communities, including reduced tourism, reduced rural and regional economic viability, loss of social amenity, and any 'side effects' of control measures.

Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)
Australia has a highly developed animal health system that can thoroughly investigate disease problems. A high priority is placed, at both national and state and territory levels, on preventing exotic animal disease incursions. Contingency planning for emergency aquatic animal diseases is well advanced at the national level. The department leads and coordinates the national management of aquatic animal health in Australia. Australia's National Strategic Plan for Aquatic Animal Health (AQUAPLAN) is jointly developed by governments and private industry sectors. Since the inception of AQUAPLAN in 1998, significant progress has been made on Australia's contemporary aquatic animal health management systems and procedures. The development of a new AQUAPLAN is underway.

AQUAVETPLAN (Australia's Aquatic Veterinary Emergency Plan) was initiated because of AQUAPLAN 1998–2003. AQUAVETPLAN is a series of manuals that outline Australia's preparedness and response plans to deal with aquatic animal disease emergencies, including a specific disease strategy manual for WSD. In addition, a committee dealing with national aquatic animal disease emergency response, the Aquatic CCEAD (Aquatic Consultative Committee on Emergency Animal Diseases), is well established. The Aquatic CCEAD has met more than 30 times and provided technical advice throughout the WSD outbreak in 2016–17.

In the Prawn IRA 2009, it was assumed that diseases that have been shown by overseas experience to be difficult or impossible to eradicate once established (for example, WSSV and YHV) would present similar difficulties in Australia. Further, the size of Australia, the difficulty of managing remote areas, the sparsity of population centres outside of the major capitals, as well as the problems of wet-season impassability of roads would further compound problems. Eradication would be very difficult or impossible if an exotic prawn hazard were to establish in Australian susceptible populations. The weight of evidence about worldwide success rates for eradicating diseases in the aquatic environment supports this assumption. Consequently, a conservative approach was taken in

this risk review, considering the high cost and time associated with attempts to eradicate new aquatic animal diseases and the challenges of success.

Environmental conditions (including husbandry) clearly influence the expression of clinical disease and the amenability of introduced disease to prevention and control. Thus, methods used successfully to respond to overseas disease events may not be feasible or similarly effective in Australia.

There would be a need for regulatory approval of any drug not registered for use with prawns in Australia if such drugs were to be used to control a newly established disease. The costs and time for registration are significant. The implementation of a control strategy, which relies on drugs to be effective, would introduce new costs and may have adverse implications for product quality and image. For some hazards, the cost of implementation of measures for control or eradication would be so high as to be unfeasible in practice.

The economic impacts of the WSD outbreak were estimated to be substantial and are ongoing in industries that operate out of the movement control zones in Queensland and New South Wales (refer section 1.5.2 Economic impact of the white spot disease outbreak for further details).

The costs of disease eradication or containment measures, including movement restrictions would affect farm profitability. For example, prawn farms would have financial losses associated with the loss of prawn stocks if diseased prawns were destroyed, the loss of production for the period the ponds were kept empty and the cost of installing additional infrastructure (for example, water filtration, pond lining, barriers for carrier exclusion.). The cost to implement extra biosecurity measures on an Australian prawn farm such as bird and crab netting, drum filtering and ozonation of water is estimated to be at least AU$1 million (Rosenberry 2017). Other estimates have put the cost to farms of establishing new biosecurity infrastructure to be approximately AU$87,600 per production pond hectare (Stephens 2017).

Economic (domestic trade effects and impact on other associated industries)

A disease outbreak may also have additional economic effects due to the loss of domestic markets, market oversupply and resulting reduction of prices received for product. Associated industries including processors, retailers, and the bait industry (for example, prawns and bloodworms) may also suffer significant production losses. Farm insurance premiums may rise, and it may be necessary to increase subsequent stocking rates to offset the effects of mortality.

Indirect impacts would also likely affect farms that are free of infection and would be most felt in those parts of Australia where crustacean farming (particularly prawn farming) makes a significant contribution to the overall local economy, such as Gold Coast, Bundaberg, Mackay, Townsville and Cairns regions of Queensland, as well as Yamba in New South Wales.

Public perception can significantly affect the markets for products intended for human consumption. This public reaction may occur irrespective of whether there is effective management of the problem, or in fact no problem at all. The use of chemical treatments or the occurrence of lesions or blemishes on the product may affect any price premiums paid for high quality products. This could occur regardless of whether the effect on quality was real or perceived. For example, WSSV can cause visible lesions in crustacean tissues, and affected product would be unacceptable to the consumer for reasons of quality and aesthetic appeal (Takahashi et al. 1994).

In general, there is no clearly documented evidence of the impact that the hazards would have in affected wild prawn fisheries. However, a reduction in the commercial wild catch would likely decrease the capacity of a fishery to support the same number of fishers. A reduction in the size of the fishery could also have commensurate impact on associated industries. Domestic trade and movement restrictions may apply to wild susceptible species fished from areas impacted by an outbreak.

It is not easy to quantify 'production' in the context of recreational fisheries. Dip-nets or two-person hand-hauled nets in local estuaries are common means to catch prawns for human consumption or use as bait. Recreational fishing for prawns and other crustacean species (such as crabs or yabbies) is a widespread fishing activity, particularly in Queensland and New South Wales. Recreational fishers in New South Wales and Australian Capital Territory harvested over 700,000 saltwater prawns and 300,000 freshwater prawns from June 2013 to May 2014 (West et al. 2015). Although spending by recreational fishers is likely to provide economic and social benefits to rural and regional areas, recreational prawn fishers represent only a few per cent of total fishers, so that (in the event of the introduction, establishment and spread of a hazard) economic losses associated with recreational prawn fishing would make a limited contribution to the total loss.

Commercial wild catch industries also include yabbies, bugs, bloodworms, beachworms and mud crabs. These industries were impacted by the WSD outbreak in South-East Queensland in 2016–17 and subsequent movement restrictions imposed for WSSV susceptible species and vectors originating from the affected area. In particular, prawns and bloodworms destined for distribution as bait and accounting for up to 80% of the Australian market were severely impacted (Commonwealth of Australia 2017).

Economic (international trade effects)

In 2020–21, Australia exported more than 4,000 tonnes of prawns (from both aquaculture and wild fisheries sectors) valued at AU$73 million (Tuynman & Dylewski 2022). The major prawn export destinations for Australia in 2020–21 were Hong Kong (1,207 tonnes valued at AU$22.6 million), Japan (685 tonnes valued at AU$16.2 million) and Vietnam (1,017 tonnes valued at AU$15.5 million) (Tuynman & Dylewski 2022).

Several countries have implemented strong import requirements or prohibited the importation of live, fresh and frozen prawns to prevent disease incursions.

Alday-Sanz (2019) reported that the Kingdom of Saudi Arabia protects its health status by banning the importation of aquatic products from countries with lower health status. Following a severe epidemic caused by WSSV in 2010, the Kingdom of Saudi Arabia prohibited import of wild broodstock. Prawn aquaculture also switched from *P. indicus* to specific pathogen free (SPF) *P. vannamei* tolerant to WSSV (Alday-Sanz 2019).

De la Peña et al. (2015) reported, that since 2013, several countries suspended or banned imports of live prawns and prawn products from countries affected by AHPND; and that the Philippines also banned imports of other crustaceans that might act as hosts of AHPND. In 2013 Costa Rica reportedly suspended the importation of crustaceans and by-products from countries affected by AHPND (Peña-Navarro et al. 2020). Likewise, Aquahoy (Aquahoy 2018) stated that Peru banned the import of prawns from regions affected by AHPND, including China, Vietnam, Malaysia, Thailand, Mexico, the

Philippines and Texas (United States of America). Kumar (2017) reported that in 2017 Thailand imposed a three-month ban on prawn imports from India over concerns about the spread of IMNV.

Japan lists several aquatic diseases subject to import quarantine for aquatic animals and aquatic animal products for aquaculture; this list includes AHPND, necrotising hepatopancreatitis (NHP), TSV, IHHNV, CMNV, YHV and GAV-disease. The European Union (EU) has in place legal requirements for the import of live prawns, which include listing of EU-approved countries and establishments, labelling to comply with traceability rules for frozen products and the presentation of a health certificate for live animals. The Republic of Korea has biosecurity requirements for imported designated crustacean species (live, frozen and chilled) to be tested for WSSV, AHPND, NHP, IHHNV, IMNV, TSV, YHV, *M. rosenbergii* nodavirus, *A. astaci* (Han et al. 2019b) and DIV1 (World Trade Organization 2020a).

Briggs et al. (2004) reported that several Central and South American countries closed their borders to the importation of live, fresh and frozen prawns after the introduction of WSSV to the region in 1999 from unknown sources. Most of those countries imposed new regulations in late 1999 (for example, Mexico) or 2000 (for example, Ecuador), which typically included specifying imports of only SPF stocks from certified, tested and enclosed facilities to certified and controlled facilities with biosecurity in the respective countries. They also insisted on PCR testing of all imported prawns for WSSV and YHV. Brazil requires that non-viable crustaceans of any origin and form must be entirely peeled, headless and gutted. Brazil has additional requirements dependent upon the commodity type.

In 2020, Taiwan notified the World Trade Organization that it was implementing emergency measures related to DIV1 for some live crustacean species, including *Cherax quadricarinatus* (World Trade Organization 2020b).

The establishment of WSSV, AHPND, YHV1, TSV, DIV1 or IMNV in Australia might have an adverse impact on export markets for Australian prawns, both live and non-viable.

If an exotic disease were to become established, Australia could use zoning to maintain access to international markets for live crustaceans including prawns and, if required, non-viable product, noting that importing countries may not necessarily accept zoning arrangements. The WOAH Code recognises the concept of zoning (regionalisation) and compartmentalisation (WOAH 2022m). Zoning would require additional specific regulatory measures such as movement restricted areas, testing and certification, with attendant costs and would be dependent on the ability to establish and maintain the zone.

Environment (biodiversity, endangered species and the integrity of ecosystems)

When evaluating the indirect impacts on the environment, the extent of harm was evaluated by considering:

- all on-site and off-site impacts
- the geographical scope and magnitude of the impact
- the frequency and duration of the action causing the harm
- the total impact which can be attributed to that action over the entire geographic area affected, and over time (that is, cumulative impact)

- reversibility of the impact; the sensitivity of the receiving environment (recognised environmental features of high sensitivity)
- the degree of confidence with which the impacts of the action are known and understood
- impacts of imbalance in ecosystems such as loss of biodiversity and integrity of the ecosystems, loss of threatened species, and whether the introduced disease was likely to endanger more common species.

The potential loss of biodiversity if a hazard were to be introduced, establish and spread, would be of concern to the Australian community. A conservative approach was taken by the department when considering the susceptibility of native species, particularly those that are endangered or threatened, to infection with the hazards. In drawing conclusions on the likely impact of exotic disease on the environment, the department considered overseas data on the species of prawns and other crustaceans that are susceptible, the effect of infection and the influence of the physical environment on the outcome of infection.

The Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) *List of threatened fauna* includes a number of crustacean species that are critically endangered, endangered or vulnerable in Australia (see Table 25) (Department of Environment and Energy 2019). The department is aware that there are other sources of information about threatened species, such as the International Union for Conservation of Nature's (IUCN) *Red list of threatened species* (IUCN 2020). Some species may be included on the *Red list of threatened species* but not on the EPBC Act's *List of threatened fauna*, such as Lecki's crayfish (*Cherax leckii*). For the purposes of this risk review, the EPBC Act's *List of threatened fauna* is considered as the authoritative list for Australian threatened species. As new species are included on the EPBC Act's *List of threatened fauna*, following assessment under the EPBC Act by the Threatened Species Scientific Committee, risk assessments may be reviewed.

Table 25 Crustacean species that are critically endangered, endangered or vulnerable in Australia

Category	Species name	Common name(s)
Critically endangered	*Cherax tenuimanus*	hairy marron, Margaret River hairy marron, Margaret River marron
	Engaewa pseudoreducta	Margaret River burrowing crayfish
	Engaewa reducta	Dunsborough burrowing crayfish
	Euastacus bindal	freshwater crayfish, spiny crayfish
	Euastacus dharawalus	Fitzroy Falls Spiny Crayfish
Endangered	*Engaeus granulatus*	Central North burrowing crayfish
	Engaeus martigener	Furneaux burrowing crayfish
	Engaeus spinicaudatus	Scottsdale burrowing crayfish
	Engaewa walpolea	Walpole Burrowing Crayfish
	Euastacus bispinosus	Glenelg Spiny Freshwater Crayfish, Pricklyback
Vulnerable	*Astacopsis gouldi*	Tasmanian giant freshwater lobster, giant lobster, giant freshwater crayfish
	Engaeus orramakunna	Mount Arthur burrowing crayfish
	Engaeus yabbimunna	Burnie Burrowing Crayfish

Social (changes in tourism, side effects from control measures, and loss of social amenity)

In the event of a disease outbreak, communities where prawn farming is a significant employer are expected to experience social impacts. Social impacts may include increased management inputs, owner stress associated with loss of livelihood and welfare concerns (including family disruptions, loss of employment and decreased living standard), impacts on businesses and industries supporting rural centres, and impacts of movement restrictions on social amenity.

Loss of social amenity by recreational fishers because of the implementation of a movement regulated area could occur. This also includes those who fish for prawns, yabbies, other crustaceans, bugs, bloodworms, beachworms and mud crabs. A reduction in recreational fishing opportunities could also result in the loss of local tourism, and consequently a loss of community income. The loss of important species for indigenous cultural fishing, like yabbies, also needs to be considered under potential social impacts.

Social impacts would be most significant in areas where crustacean aquaculture, particularly prawn farming plays a major role in the local economy, for example the Gold Coast, Bundaberg, Mackay, Townsville, and Cairns in Northern Queensland and Yamba in New South Wales.

Appendix E: Minor exposure pathways

The Prawn IRA 2009 identified several minor exposure pathways. These exposure pathways have a much lower probability of completion because inactivation of the hazard occurs before potential exposure or they involve only indirect exposure of the aquatic environment. These pathways were not considered further when conducting the risk assessments for this risk review.

Disposal of solids and liquid waste from commercial processing of imported prawns

Prior to the Prawn IRA 2009, there were minimal biosecurity requirements for processing of imported whole prawns in Australia. Therefore, disposal of solids and liquid waste from commercial processing of imported prawns was considered a major pathway in the Prawn IRA 2009 (Biosecurity Australia 2009).

However, under the current import conditions uncooked prawns which have had the head and shell removed and which do not pass on-arrival virus testing, are not permitted to be further processed (for example, cooked) unless within an approved arrangement (AA). Although it is noted that this option is seldom utilised and batches which do not pass on-arrival virus testing are generally re-exported, although destruction is also an option provided to the importer. Breaded, battered and crumbed prawns must be sold in their imported form and must not be altered in any way, further processed or repackaged without written approval from the department. Approved arrangements set out the requirements for undertaking activities. Some requirements are specific to the class of AA and some apply across multiple classes. The type of activity taking place in the AA and the associated biosecurity risks determines the class of AA. Class 3.3 – Imported uncooked prawn product processing allows for processing of imported prawns. This ensures management of all associated biosecurity risks, including disposal of wastewater and solids. The department notes that despite there being an appropriate AA class for processing of imported prawns, it is rarely utilised and no processing of imported prawns currently occurs in Australia on a regular basis. This is likely due to there being minimal financial benefit to process imported prawns onshore.

If imported prawns were processed outside of an AA this could substantially contribute to the risk of untreated biosecurity waste entering the natural environment. While non-compliance with import conditions is outside the scope of this risk review, the department applies a range of regulatory tools to manage compliance. This includes routine inspections and audits, through to criminal prosecution. The Biosecurity Compliance Statement outlines the department's approach to managing compliance with biosecurity conditions. Additionally, the Biosecurity Act 2015 provides the department with regulatory tools to help identify, manage and respond to non-compliance and biosecurity risk. Despite reports and investigations, the department is yet to uncover unapproved processing of imported prawns in Australia.

Due to changes in import conditions that have occurred since the Prawn IRA 2009 was released, this exposure pathway is considered a minor pathway in this risk review.

Human consumption

Human consumption is the primary purpose for which prawns are imported. Of the hazards identified in this risk review, it is expected that the amount of viable hazard would be dramatically reduced or eliminated, in the human gastrointestinal tract. Additionally, in Australia, human faecal wastes are normally disposed of via domestic sewerage systems.

The Prawn IRA 2009 considered that the physico-chemical environment of such systems, combined with the effect of dilution with other wastes, would reduce substantially both the level and concentration of any remaining aquatic animal pathogens. As such, prawns eaten by humans would not contribute significantly to the biosecurity risk of imported prawns (Biosecurity Australia 2009). This conclusion is still valid and this pathway is not considered a major exposure pathway in this risk review.

Use of imported prawns in the manufacture of pelletised feed for crustacean aquaculture

The Prawn IRA 2009 determined that in the event prawns imported for human consumption, or their associated wastes, were used in Australia for manufacturing pelleted aquaculture feeds, that the heat treatments associated with feed manufacture would substantially, if not completely, inactivate any prawn pathogens present (Biosecurity Australia 2009). This conclusion is still valid and this pathway is not considered a major exposure pathway in this risk review.

Prawn waste disposed at controlled landfill sites

The Prawn IRA 2009 identified that the environmental conditions at landfill sites would likely result in the exposure of any aquatic animal pathogens present to desiccation, ultraviolet radiation, low oxygen potential, daily variations in temperature, or competition from other microorganisms for nutrients. Such exposures are expected to reduce the amount of any hazard present or, in some cases, may eliminate the hazard entirely (Biosecurity Australia 2009). This conclusion is still valid and this pathway is not considered a major exposure pathway in this risk review.

Food scraps discarded directly into the aquatic environment

Food scraps directly discarded into the aquatic environment may be an exposure pathway. Food scraps are considered to be the remains of prawns or prawn products, following a meal. It is considered that the bulk of the muscle is eaten and the products cooked prior to consumption.

The Prawn IRA 2009 considered that infection of susceptible prawns or other host animals due to the discarding of food scraps into the aquatic environment would be unlikely. Most of the scraps were considered to be cooked and unlikely to contain hazards in infective form or in high concentrations. Moreover, discarded scraps were more likely to be consumed by non-susceptible rather than susceptible species (Biosecurity Australia 2009). This conclusion is still valid and this pathway is not considered a major exposure pathway in this risk review.

The Kantar Public survey reported that 6% of fishers surveyed had used ‘left-over’ cooked prawns from a meal as fishing bait (Kantar Public 2017). That is they cooked prawns for human consumption and used the ‘left-overs’ as bait. Whilst ‘left-over’ was not fully defined in the Kantar Public survey, it is assumed that the bulk of the muscle tissue, shell and head (for whole prawns) is intact for ‘left-

over' cooked prawns. The bait and berley exposure pathway takes into account the use of 'left-over' prawns.

Prawn wastes disposed through municipal sewerage systems

The Prawn IRA 2009 considered that the processing of effluent in a domestic sewerage system would, even if it were limited to primary level processing, significantly reduce, if not eliminate, the concentration of any prawn pathogens that might be present. At a minimum, the physical and biological treatment, disinfected secondary treatment (chlorination) and dilution of effluent in most Australian sewerage systems is capable of eliminating hazards prior to discharge (Biosecurity Australia 2009). This conclusion is still valid and this pathway is not considered a major exposure pathway in this risk review.

Discharge of processing effluent into freshwater

The Prawn IRA 2009 considered that the discharge of processing effluent into freshwater is usually controlled by local authorities that normally require processing to a secondary or tertiary level to protect public health and the environment. Such processing would reduce the concentration of hazards entering freshwater systems by several orders of magnitude (Biosecurity Australia 2009). This conclusion is still valid and it is also considered that this exposure pathway is encompassed under the Disposal of solids and liquid waste from commercial processing of imported prawns exposure pathway. An AA is required for processing of imported prawns in Australia and the conditions under the AA would manage biosecurity risk. This pathway is not considered a major exposure pathway in this risk review.

Other minor pathways

The Prawn IRA 2009 identified several other possible, but unlikely, exposure pathways. These pathways included diversion of prawns for use as agricultural fertiliser, disposal of packaging materials used in importation of whole uncooked prawns, use of imported prawns as an ingredient in animal feed manufacture (other than use in manufacture of crustacean aquaculture feeds) and chitin production, and use of imported prawns as feed for display animals kept in home aquaria. There may also be other minor potential pathways by which susceptible host animals in Australia are exposed to imported prawns (or associated wastes).

Appendix F: Risk assessment values for unrestricted and restricted import of prawns

Table 26 shows the risk assessment values for unrestricted import and restricted (biosecurity measures applied) import for each hazard.

Table 26 Risk assessment values for unrestricted risk of import and with biosecurity measures applied for each hazard

Hazard	Biosecurity measure	Likelihood of entry	Partial likelihood of exposure			Partial annual likelihood of entry and exposure			Partial likelihood of establishment and spread			Impact								Likely consequences			Partial annual risk			Annual risk
			Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect – social	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	All crustaceans
"Ca. H. penaei"	**Unrestricted (frozen)**	**VL**	**EL**	**EL**	**VL**	**EL**	**EL**	**EL**	**M**	**L**	**L**	**M**	**D**	**A**	**E**	**D**	**C**	**A**	**B**	**M**	**L**	**L**	**N**	**N**	**N**	**N**
	Unrestricted (chilled)	H	VL	L	M	VL	L	M	M	L	L	M	D	A	E	D	C	A	B	M	L	L	VL	VL	L	L
CMNV	**Unrestricted**	**H**	**VL**	**L**	**M**	**VL**	**L**	**M**	**M**	**L**	**VL**	**M**	**E**	**A**	**E**	**D**	**B**	**A**	**B**	**M**	**L**	**VL**	**VL**	**VL**	**VL**	**L**
	H&S removal	M	EL	VL	L	EL	VL	L	M	L	VL	M	E	A	E	D	B	A	B	M	L	VL	N	N	N	N
	Cooking	M	EL	N	L	EL	N	L	M	L	VL	M	E	A	E	D	B	A	B	M	L	VL	N	N	N	N
	VAP	H	N	N	EL	N	N	EL	M	L	VL	M	E	A	E	D	B	A	B	M	L	VL	N	N	N	N
DIV1	**Unrestricted**	**H**	**VL**	**L**	**H**	**VL**	**L**	**H**	**H**	**L**	**L**	**M**	**E**	**B**	**E**	**D**	**C**	**E**	**B**	**M**	**L**	**L**	**VL**	**VL**	**L**	**L**
	H&S removal	M	EL	VL	M	EL	VL	L	H	L	L	M	E	B	E	D	C	E	B	M	L	L	N	N	VL	VL
	Cooking	M	EL	N	M	EL	N	L	H	L	L	M	E	B	E	D	C	E	B	M	L	L	N	N	VL	VL
	VAP	H	N	N	EL	N	N	EL	H	L	L	M	E	B	E	D	C	E	B	M	L	L	N	N	N	N

Hazard	Biosecurity measure	Likelihood of entry	Partial likelihood of exposure			Partial annual likelihood of entry and exposure			Partial likelihood of establishment and spread			Impact								Likely consequences			Partial annual risk			Annual risk
			Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect – social	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	All crustaceans
EHP	**Unrestricted**	**M**	**VL**	**L**	**M**	**VL**	**L**	**L**	**M**	**L**	**M**	**M**	**E**	**A**	**E**	**D**	**B**	**A**	**B**	**M**	**L**	**M**	**VL**	**VL**	**L**	**L**
	H&S removal	M	EL	EL	L	EL	EL	L	M	L	M	M	E	A	E	D	B	A	B	M	L	M	N	N	L	L
	H&S removal + devein	L	EL	EL	L	EL	EL	VL	M	L	M	M	E	A	E	D	B	A	B	M	L	M	N	N	VL	VL
	Cooking	L	EL	EL	L	EL	N	VL	M	L	M	M	E	A	E	D	B	A	B	M	L	M	N	N	VL	VL
	VAP	M	N	N	EL	N	N	EL	M	L	M	M	E	A	E	D	B	A	B	M	L	M	N	N	N	N
IMNV	**Unrestricted**	**H**	**VL**	**L**	**M**	**VL**	**L**	**M**	**M**	**L**	**VL**	**M**	**C**	**A**	**E**	**D**	**C**	**A**	**B**	**M**	**L**	**VL**	**VL**	**VL**	**VL**	**L**
	H&S removal	H	EL	VL	L	EL	VL	L	M	L	VL	M	C	A	E	D	C	A	B	M	L	VL	N	N	N	N
	Cooking	H	EL	N	L	EL	N	L	M	L	VL	M	C	A	E	D	C	A	B	M	L	VL	N	N	N	N
	VAP	H	N	N	EL	N	N	EL	M	L	VL	M	C	A	E	D	C	A	B	M	L	VL	N	N	N	N
LSNV	**Unrestricted**	**H**	**VL**	**L**	**M**	**VL**	**L**	**M**	**M**	**L**	**VL**	**M**	**D**	**A**	**E**	**D**	**B**	**A**	**B**	**M**	**L**	**VL**	**VL**	**VL**	**VL**	**L**
	H&S removal	M	EL	VL	L	EL	VL	L	M	L	VL	M	D	A	E	D	B	A	B	M	L	VL	N	N	N	N
	Cooking	H	EL	N	L	EL	N	L	M	L	VL	M	D	A	E	D	B	A	B	M	L	VL	N	N	N	N
	VAP	M	N	N	EL	N	N	EL	M	L	VL	M	D	A	E	D	B	A	B	M	L	VL	N	N	N	N
TSV	**Unrestricted**	**H**	**VL**	**L**	**M**	**VL**	**L**	**M**	**M**	**L**	**VL**	**M**	**D**	**B**	**E**	**D**	**C**	**E**	**B**	**M**	**L**	**VL**	**VL**	**VL**	**VL**	**L**
	H&S removal	H	EL	VL	L	EL	VL	L	M	L	VL	M	D	B	E	D	C	E	B	M	L	VL	N	N	N	N
	Cooking	H	EL	N	L	EL	N	L	M	L	VL	M	D	B	E	D	C	E	B	M	L	VL	N	N	N	N
	VAP	H	N	N	EL	N	N	EL	M	L	VL	M	D	B	E	D	C	E	B	M	L	VL	N	N	N	N

Hazard	Biosecurity measure	Likelihood of entry	Partial likelihood of exposure			Partial annual likelihood of entry and exposure			Partial likelihood of establishment and spread			Impact								Likely consequences			Partial annual risk			Annual risk
			Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect – social	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	All crustaceans
Vp AHPND	**Unrestricted**	**VL**	**EL**	**EL**	**L**	**EL**	**EL**	**VL**	**H**	**L**	**M**	**H**	**F**	**A**	**E**	**E**	**C**	**E**	**B**	**H**	**M**	**H**	**VL**	**N**	**L**	**L**
	H&S removal	EL	N	N	VL	N	N	EL	H	L	M	H	F	A	E	E	C	E	B	H	M	H	N	N	VL	VL
	Cooking	EL	N	N	VL	N	N	EL	H	L	M	H	F	A	E	E	C	E	B	H	M	H	N	N	VL	VL
	VAP	EL	N	N	EL	N	N	N	H	L	M	H	F	A	E	E	C	E	B	H	M	H	N	N	N	N
WSSV	**Unrestricted**	**H**	**VL**	**M**	**H**	**VL**	**M**	**H**	**H**	**M**	**H**	**H**	**F**	**D**	**E**	**E**	**C**	**E**	**C**	**H**	**H**	**H**	**L**	**H**	**H**	**E**
	H&S removal	H	EL	L	M	EL	L	M	H	M	H	H	F	D	E	E	C	E	C	H	H	H	VL	M	H	H
	H&S removal + devein	H	EL	L	M	EL	L	M	H	M	H	H	F	D	E	E	C	E	C	H	H	H	VL	M	H	H
	H&S removal + testing	L	EL	L	M	EL	VL	L	H	M	H	H	F	D	E	E	C	E	C	H	H	H	VL	L	M	M
	H&S removal + 2x testing	EL	EL	L	M	N	EL	EL	H	M	H	H	F	D	E	E	C	E	C	H	H	H	N	VL	VL	VL
	Cooking	EL	EL	EL	M	N	N	EL	H	M	H	H	F	D	E	E	C	E	C	H	H	H	N	N	VL	VL
	VAP	H	N	N	EL	N	N	EL	H	M	H	H	F	D	E	E	C	E	C	H	H	H	N	N	VL	VL
YHV1	**Unrestricted**	**H**	**VL**	**L**	**H**	**VL**	**L**	**H**	**H**	**L**	**L**	**H**	**F**	**B**	**E**	**E**	**C**	**E**	**C**	**H**	**M**	**M**	**L**	**L**	**M**	**M**
	H&S removal	H	EL	VL	M	EL	VL	M	H	L	L	H	F	B	E	E	C	E	C	H	M	M	VL	VL	M	M
	H&S removal + devein	H	EL	VL	M	EL	VL	M	H	L	L	H	F	B	E	E	C	E	C	H	M	M	VL	VL	M	M
	H&S removal + testing	L	EL	VL	M	EL	VL	L	H	L	L	H	F	B	E	E	C	E	C	H	M	M	VL	VL	L	L

Hazard	Biosecurity measure	Likelihood of entry	Partial likelihood of exposure			Partial annual likelihood of entry and exposure			Partial likelihood of establishment and spread			Impact								Likely consequences			Partial annual risk			Annual risk
			Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect – social	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	Farmed crustaceans	Hatchery crustaceans	Wild crustaceans	All crustaceans
	H&S removal + 2x testing	EL	EL	VL	M	N	EL	EL	H	L	L	H	F	B	E	E	C	E	C	H	M	M	N	N	N	N
	Cooking	EL	EL	N	M	N	N	EL	H	L	L	H	F	B	E	E	C	E	C	H	M	M	N	N	N	N
	VAP	H	N	N	EL	N	N	EL	H	L	L	H	F	B	E	E	C	E	C	H	M	M	N	N	N	N

Hazards: "Ca. H. penaei " "Candidatus Hepatobacter penaei". **CMNV** covert mortality nodavirus. **DIV1** decapod iridescent virus 1. **EHP** *Enterocytozoon hepatopenaei*. **IMNV** infectious myonecrosis virus. **LSNV** Laem-Singh virus. **TSV** Taura syndrome virus. **Vp AHPND** *Vibrio parahaemolyticus* strains containing Pir toxins. **WSSV** white spot syndrome virus. **YHV1** yellow head virus genotype 1. **Biosecurity measures: Unrestricted** no biosecurity measures applied. **H&S removal** head and shell removal. **H&S removal + devein** head and shell removal plus deveining. **H&S removal + testing** head and shell removal in combination with pre-export testing. **H&S removal + 2x testing** head and shell removal in combination with pre-export and on-arrival testing. **Cooking** with the requirement that the prawns appear fully cooked and have achieved a core temperature of at least 65°C. **VAP** value-added product. **Risk rating: E** Extreme. **H** High. **M** Moderate. **L** Low. **VL** Very low. **EL** Extremely low. **N** Negligible. **Impact score (A, B, C, D, E, F):** See Figure 4. **All crustaceans:** farmed, hatchery and wild crustacean exposure groups combined.

Appendix G: Legislation, policies and guidelines related to prawn biosecurity

Each Australian state and territory has its own legislation related to fisheries, and requirements vary across jurisdictions. Some states and territories have legislation that could apply in cases of deliberate introduction of exotic pests or diseases into the aquatic environment. However, this legislation is not easily or readily enforceable.

Summary of relevant legislation for each state and territory

Australian Capital Territory

The Australian Capital Territory (ACT) did not provide information for this risk review. The department notes that the ACT does not have a prawn industry.

New South Wales

Movement of broodstock and postlarvae into New South Wales (NSW) for stocking into NSW farms is managed through a Health protocol for translocation of prawn post-larvae into NSW for stocking into NSW prawn farms for the 2021 season. NSW does not have any restrictions on movements of live prawns within NSW.

New South Wales does not have specific regulations preventing the use of prawns intended for human consumption being used as bait or berley. Advisory materials encouraging fishers to not use prawn meat for human consumptions as bait have been provided to fishers. Part 3 of the Biosecurity Act 2015 (NSW) provides General Biosecurity Duty requirements, however they apply where specific risks have been identified and compliance is difficult to enforce given the extremely large number of recreational fishers in NSW. The NSW Fisheries Management (General) Regulation 2019 prohibits the use of bait in the freshwater environment that is not native to the waters of NSW (other than dead carp). However, it is understood that enforcement of compliance is challenging and it is understood that marine prawns are commonly used as bait by freshwater fishers in NSW.

New South Wales does not have legislation, policies or guidelines in relation to the use of prawns (or other seafood), intended for human consumption, as feed in commercial, research or public display aquaria.

Northern Territory

The Northern Territory did not provide information for this risk review. The department notes that holders of licences issued in accordance with section 11 of the Northern Territory Fisheries Act 1988 can have restrictions placed on the licence that prohibit the use of uncooked imported prawns as bait or aquaculture feed.

Queensland

Queensland have put in place the Health protocol for the movement of live prawns which applies to all prawns caught for the purposes of being used as broodstock in the prawn farming sector. This protocol also manages the movement of live prawns into and within Queensland.

Queensland does not have recreational fishing licenses and no specific conditions are applicable to using prawns or other seafood products intended for human consumption as bait or berley. Under section 91 of the Queensland Fisheries Act 1994, a person must not unlawfully release aquaculture fisheries resources, or cause aquaculture fisheries resources to be released into Queensland waters. Sections 90 and 91 of the Fisheries Act 1994 defines criteria for non-indigenous and aquaculture fisheries resources where a person must not unlawfully release non-indigenous fisheries resources or cause non-indigenous fisheries resources to be placed or released, into Queensland waters. It restricts using non-indigenous fisheries resources as live bait, for example red claw crayfish may not be used in areas where it is not indigenous.

Following the white spot disease (WSD) outbreak, the Queensland Government implemented fishing restrictions around all prawn farms in the Logan River region. Line fishing was prohibited within 100 metres of prawn farm water intake and outlet channels and all fishing in drainage channels surrounding these prawn farms was prohibited. Crab pots, cast nets and yabby pumps were permitted to be used in waterways adjacent to prawn farms, unless signage stated otherwise. Raw prawns, yabbies and marine worms could not be moved out of the WSD movement regulated area. This measure is still in place at the time this report was prepared and is not applicable to the whole of the State.

To protect Queensland's natural waterways and prevent disease spread, it is a condition of an aquaculture development approval that aquaculture fisheries resources must not be sold, traded or given away for the purpose of being used as bait. There are exemptions in place for freshwater prawns, blood worms and sand wriggler worms.

Queensland does not have legislation, policies or guidelines in relation to the use of prawns (or other seafood), intended for human consumption, as feed in commercial, research or public display aquaria.

South Australia

South Australia has legislation prohibiting the use of animals other than fish, worms or insects as berley within two nautical miles of the State (section 24 of the Fisheries Management (General) Regulations 2017). There are exceptions in place for use of animals as bait in rock lobster pots or fish traps. The regulation also applies to any island or reef exposed at the low water mark and that forms part of South Australia. Further, it is an offense to release or deposit exotic and/or aquaculture farmed species into the waters of South Australia (section 78 of the Fisheries Management Act 2007). Section 29 of the Livestock Act 1997 states that a person must not bring into South Australia a notifiable disease or cause a notifiable disease to be brought into the State. To protect its natural waterways and help prevent disease spread the South Australian Government has provided fish processors, fisheries and aquaculture associations with information informing them of legislation and policy in relation to bait and berley use. This included the *National policy guidelines for translocation of domestic bait and berley* which captures the risk of using seafood, intended for human consumption, as bait or berley.

Following the WSD outbreak, South Australia implemented import restrictions for prawns and other crustaceans including polychaetes that originated in the WSD movement regulated area. Prawns from the WSD movement regulated area that were either cooked or gamma irradiated before entering South Australia are exempt from the ban. Some other live high-value decapods (such as,

mud crabs and Balmain bugs) are recognised as being low risk and are permitted entry under strict conditions, including appropriate disposal of product and waste.

In relation to the use of prawns (or other seafood), intended for human consumption, as feed in commercial, research or public display aquaria, that product must not be used as feed if it may cause livestock to become affected with a notifiable condition (Section 32 of the Livestock Act 1997). Livestock means animals kept or usually kept in a domestic or captive state including fish (by definition within the Livestock Act 1997 means all aquatic animals other than mammals, such as crustaceans) kept or usually kept in an aquarium or fish farm.

Tasmania

Tasmania did not provide information for this risk review. The department notes that Tasmania does not have a prawn industry, however it does have vulnerable native crustacean populations and a significant rock lobster industry.

Victoria

Victoria does not have a prawn industry. Commercial and recreational fishing for prawns and other crustaceans are managed under the Fisheries Regulations 2019. The regulations are made under the Victorian Fisheries Act 1995. Recreational fishing is subject to catch and gear limits, whilst commercial wild catch fishing are subject to limited licences, closed areas and gear restrictions. Section 138 and 139 of the Fisheries Regulations 2019 identify bait types not to be used in Victorian waters. The use of crustacean species, other than live European green shore crab, is not restricted in Victorian waters.

Victoria does not have legislation, policies or guidelines in relation to the use of prawns (or other seafood), intended for human consumption, as feed in commercial, research or public display aquaria.

Western Australia

Western Australia regulates aquaculture activities using aquaculture licences in accordance with the Fish Resources Management Act 1994 and the Fish Resources Management Regulations 1995. The disease risks of moving live crustaceans is managed through movement conditions for aquaculture licences or for live animal translocations in accordance with the Biosecurity and Agriculture Management Act 2007 and the Fish Resources Management Regulations 1995. Significant diseases of crustaceans that are considered exotic to Western Australia are listed as reportable/notifiable under Western Australian legislation and are prohibited organisms, including decapod penstyldensovirus 1 (currently known as decapod penstylhamaparvovirus 1, and previously known as infectious hypodermal and haematopoietic necrosis virus, IHHNV). Western Australia has established disease zones for gill associated virus and lymphoid organ virus.

Following the WSD outbreak, Western Australia implemented import restrictions to decapod crustaceans and polychaetes that are produced by aquaculture in Queensland or New South Wales, as well as crustaceans wild-caught in Queensland from a restriction area. Precautionary enhanced restrictions now also apply to the import of bait prawns from NSW.

Western Australia does not have specific regulations preventing the use of prawns intended for human consumption being used as bait or berley. However, Western Australia has undertaken

communication activities to promote not using uncooked prawns intended for human consumption as bait, not disposing of prawn waste in or near waterways and reporting any signs of diseases.

Aquaculture licences in Western Australia include conditions to manage the risk of disease introduction to the facility, which generally includes conditions on the source and type of feed this is permitted for use (where relevant).

Western Australia does not have specific legislation or published guidelines in relation to the use of prawns (or other seafood), intended for human consumption, as feed in commercial, research or public display aquaria.

Glossary

Term or abbreviation	Definition
Approved arrangement (AA)	Approved arrangement (AA) is defined in the Biosecurity Act 2015 as an arrangement for which an approval is in force under paragraph 406(1)(a) (including a varied arrangement for which an approval is in force under that paragraph as it applies because of subsection 412(3)).
ABARES	Australian Bureau of Agricultural and Resource Economics and Sciences
AHPND	Acute hepatopancreatic necrosis disease
appropriate level of protection (ALOP) for Australia	The Biosecurity Act 2015 defines the appropriate level of protection (or ALOP) for Australia as a high level of sanitary and phytosanitary protection aimed at reducing biosecurity risks to very low, but not to zero.
Aquatic CCEAD	Aquatic Consultative Committee on Emergency Animal Diseases. The Aquatic CCEAD is a coordinating body providing the technical link between industry, the Australian Government, and state and territory governments for decision making during aquatic animal health emergencies.
BICON	Australia's Biosecurity Import Condition System
biosecurity	The prevention of the entry, establishment or spread of unwanted pests and infectious disease agents to protect human, animal or plant health or life, and the environment.
biosecurity import risk analysis (BIRA)	The Biosecurity Act 2015 defines a BIRA as an evaluation of the level of biosecurity risk associated with particular goods, or a particular class of goods, that may be imported, or proposed to be imported, into Australian territory, including, if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or the class of goods, to a level that achieves the ALOP for Australia. The risk analysis process is regulated under legislation.
biosecurity measures	The Biosecurity Act 2015 defines biosecurity measures as measures to manage biosecurity risk, the risk of contagion of a listed human disease, the risk of listed human diseases entering, emerging, establishing themselves or spreading in Australian territory, and biosecurity emergencies and human biosecurity emergencies.
biosecurity risk	The Biosecurity Act 2015 refers to biosecurity risk as the likelihood of a disease or pest entering, establishing or spreading in Australian territory, and the potential for the disease or pest causing harm to human, animal or plant health, the environment, economic or community activities.
compartment	One or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status with respect to a specific disease(s) for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purpose of international trade. Such must be clearly documented by the competent authority.
competent authority	The Veterinary Authority or other Government Authority of a Member Country having the responsibility and competence for ensuring or supervising the implementation of aquatic animal health and welfare measures, international health certification and other standards and recommendations in the OIE Aquatic Code in the whole territory.
country freedom	Within a country, the absence of a certain disease under consideration where requirements, as specified in the World Organisation for Animal Health (WOAH, formerly OIE) *Aquatic animal health code*, or determined by the Department of Agriculture, Fisheries and Forestry have been demonstrated.
CMNV	covert mortality nodavirus

Term or abbreviation	Definition
DIV1	decapod iridescent virus 1 (DIV1)
DNA	deoxyribonucleic acid
EHP	*Enterocytozoon hepatopenaei*
equivalence	The state wherein the biosecurity measure(s) proposed by the exporting country as an alternative to those of the importing country, achieve(s) the same level of protection as those prescribed by the importing country.
endemic	Belonging to, native to, or prevalent in a particular geography, area or environment.
exotic	When referring to a disease, is not present in the country of concern, and for which measures are in place to either prevent or detect possible incursion of the disease into the country.
FAO	Food and Agriculture Organization of the United Nations.
FSANZ	Food Standards Australia New Zealand.
goods	The Biosecurity Act 2015 defines goods as an animal, a plant (whether moveable or not), a sample or specimen of a disease agent, a pest, mail or any other article, substance or thing (including, but not limited to, any kind of moveable property).
HACCP	Hazard Analysis Critical Control Point - a system that identifies, evaluates and controls hazards that are significant for food safety.
hazard	A biological, chemical or physical agent in, or a condition of, an aquatic animal or aquatic animal product with the potential to cause an adverse effect on aquatic animal health or public health.
hazard identification	Identification of potential hazards that may be associated with the importation of a commodity.
health certificate	For an animal or part of an animal that is to be imported into Australian territory from a place outside Australian territory (the overseas place) means a certificate that is in a form approved by the Director of Biosecurity and has been signed by an approved officer from the overseas place.
IHHNV	Decapod penstylhamaparvovirus 1 (formerly known as decapod penstyldensovirus 1 and infectious hypodermal and haematopoietic necrosis virus (IHHNV))
IMNV	infectious myonecrosis virus
import permit	Official document authorising a person to bring or import particular goods into Australian territory in accordance with specified import requirements.
IRA	import risk analysis
LSNV	Laem-Singh virus
mins	minutes
MSGS	monodon slow growth syndrome
NHP	necrotising hepatopancreatitis
non-regulated risk analysis	Refers to the process for conducting a risk analysis that is not regulated under legislation (Biosecurity import risk analysis guidelines 2016).
official control program	A program which is approved, and managed or supervised by the Veterinary Authority of a Member Country for the purpose of controlling a pathogen or disease by specific measures applied throughout that Member Country, or within a zone or compartment of that Member Country.
OIE	World Organisation for Animal Health (WOAH, formerly OIE)

Term or abbreviation	Definition
PCR	polymerase chain reaction
per os	Describes a treatment that is taken orally. From Latin 'by opening' or 'by way of the opening'
prawn	Decapod of suborder Dendrobranchiata (Decapoda) and infraorder Caridea (Pleocyemata: Decapoda).
Prawn IRA 2009	Generic import risk analysis report for prawns and prawn products 2009
qPCR	Quantitative (real-time) polymerase chain reaction
qRT-PCR	Quantitative (real-time) reverse-transcription-polymerase chain reaction
restricted risk	Risk estimate with biosecurity measure(s) applied.
risk analysis	Refers to the technical or scientific process for assessing the level of biosecurity risk associated with the goods, or the class of goods, and if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or class of goods to a level that achieves the ALOP for Australia.
risk assessment	The scientific evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a hazard.
risk management	The process of identifying, selecting and implementing measures that can be applied to reduce the level of risk.
RNA	ribonucleic acid
RT-LAMP	reverse transcription loop-mediated isothermal amplification
RT-PCR	Reverse-transcription-polymerase chain reaction
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures.
the department	The Australian Government Department of Agriculture, Fisheries and Forestry
TSV	Taura syndrome virus
unrestricted risk	Risk estimate without the application of biosecurity measures.
Vp AHPND	*Vibrio parahaemolyticus* strains containing Pir toxins
WOAH	World Organisation for Animal Health (WOAH, formerly OIE)
WSD	White spot disease
WSSV	White spot syndrome virus
WTO	World Trade Organization
YHV	Yellow head virus
zone	An area in one or more countries containing an aquatic animal population with a specific aquatic animal health status with respect to a disease, in which surveillance and control measures and basic biosecurity conditions are applied. The zone should be defined by the Competent Authority.

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