

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

## Draft report

September 2020



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#### Acknowledgements

The authors wish to thank the subject matter experts who provided expert opinion on this draft review.

#### Stakeholder submissions on draft reports

This draft report allows interested parties to comment on relevant technical biosecurity issues. A final report will consider any comments received.

Submissions should be sent to the Department of Agriculture, Water and the Environment and must meet the conditions specified in the relevant <u>Animal Biosecurity Advice</u>.

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## Update on recreational fishing surveys

The *Generic import risk analysis report for prawns and prawn products 2009* (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. There have not been national surveys conducted since that time.

The Australian Bureau of Agricultural and Resource Economics and Sciences, the University of Canberra and Kewagama Research are now conducting the *National social and economic recreational fishing survey*. This survey will collect current 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. Information obtained will include data on whether cooked prawns, uncooked prawns which have had the head and shell removed and highly processed prawns are used as bait or berley by recreational fishers. The *National social and economic recreational fishing survey* is targeting 4,000 to 6,000 respondents across Australia to allow for an accurate representation of the Australian population. The outputs of the *National social and economic recreational fishing survey* will be validated against probability-based concurrent state-wide surveys.

The Australian Government Department of Agriculture, Water and the Environment expected to have preliminary data from the *National social and economic recreational fishing survey*, as it relates to the use of prawns as bait and berley in early-2020, for consideration and inclusion when preparing this draft report. However, the catastrophic bushfires experienced over the summer period impacted participants in the survey. Because suitable participant numbers for statistically robust data to be obtained was not reached, the closing date was extended from mid-February 2020 to mid-May 2020. The survey has also been affected by the COVID-19 lockdown, with the impacts currently being assessed. Due to these delays in the survey deadline, with analysis and validation still to come, the data on the use of prawns as bait or berley by recreational fishers is not available for inclusion in this draft risk review.

Rather than delay release of this draft report, the department has decided to release the report using the assumptions outlined within which were made based on available data and are suitably conservative. The outcomes of the *National social and economic recreational fishing survey* and the way in which they affect the overall conclusions of this draft risk review will be released when the data becomes available and have been analysed. There may be a further public consultation period at that time.

The department would like to highlight that the most likely part of the risk assessments which this data may affect is the exposure of wild crustaceans to imported prawns, especially prawns which have had the head and shell removed. This is because the department has taken a conservative approach (based on the available information) and estimated exposure of wild crustaceans to this product type to be likely. It is noted that the department does not believe that a reduction in exposure likelihood for the wild crustacean exposure group will change the overall risk estimation for most hazards.

## **Summary**

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

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

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

This draft risk review identifies hazards that require biosecurity measures to manage the risks to a very low level in order to achieve Australia's appropriate level of protection (ALOP). The hazards requiring biosecurity measures are:

- "Candidatus Hepatobacter penaei" (chilled product only)
- 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 genotype 1.

This draft risk review proposes a combination of biosecurity measures to achieve Australia's ALOP, specifically:

- sourcing from free populations
- cooking
- freezing
- value-added products which encompasses breaded, battered and crumbed prawns, and dumpling and dim sum type-products containing uncooked prawns
- head and shell removal (last tail segment and tail fans permitted)
- deveining (removal of the digestive tract to at least the last shell segment)
- batch testing for hazards
- labelling for human consumption-only.

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 draft report. Submissions supporting equivalence measures will be considered on a case-by-case basis.

This draft report contains details of the risk review for each hazard and the proposed biosecurity measures to manage identified risks. Interested parties can provide comments and submissions to the department within the consultation period.

## 1 Introduction

#### **1.1** Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests and diseases entering, establishing and spreading in Australia. Exotic pests and diseases threaten Australia's unique flora and fauna, agricultural industries and human health.

The risk analysis process is an important part of Australia's biosecurity policies. 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.

The Australian Government Department of Agriculture, Water and the Environment 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 take the form of a biosecurity import risk analysis or a non-regulated risk analysis (such as review of existing policy and import conditions (risk review), or scientific advice). Further information about Australia's biosecurity framework is provided in the *Biosecurity import risk analysis guidelines 2016* located on the department's <u>website</u>.

#### **1.2** This draft risk review

#### 1.2.1 Background

The department released <u>Biosecurity Advice 2017-07</u> 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 and in recognition of emerging/new 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.

The suspension ended on 6 July 2017 and interim enhanced import conditions as outlined in <u>Biosecurity Advice 2017-12</u> (Department of Agriculture and Water Resources 2017b) and <u>Biosecurity Advice 2018-15</u> (Department of Agriculture and Water Resources 2018b) were put in place to manage the biosecurity risks. During completion of this risk review, the department

identified that the import conditions outlined in <u>Biosecurity Advice 2017-12</u> and <u>Biosecurity</u> <u>Advice 2018-15</u> did not manage the biosecurity risks associated with the hazard *Enterocytozoon hepatopenaei* (EHP). On 14 May 2020 the department released <u>Animal</u> <u>Biosecurity Advice 2020-A03</u> notifying stakeholders of the implementation of interim import conditions that require all uncooked prawns imported for human consumption to be deveined (Department of Agriculture, Water and the Environment 2020). Implementation of the deveining requirement occurred on 1 July 2020.

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.

#### 1.2.2 Scope

The scope of this draft risk review is to consider the biosecurity risk associated with the import of prawns from all countries for human consumption. The 'unrestricted commodity' (or singleentry 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 draft 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 whole, 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 draft 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 draft 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 undergone1. 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. Additionally, 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 draft 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. Recognition of individual country disease status and sourcing from wild fisheries for export are biosecurity measures considered separately.

Prawns (also known as shrimp) are considered to be 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 draft risk review.

#### 1.2.3 Existing policy

#### Import policy

Import policy exists for prawns from those countries approved by the department to export prawns to Australia. The department has progressively changed the import requirements for imported prawns since July 2017.

The current import requirements for prawns are on the <u>department's website</u> in <u>Animal</u> <u>Biosecurity Advice 2020-A03</u> (Department of Agriculture, Water and the Environment 2020) and on the <u>Australian Biosecurity Import Conditions</u> (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

On 26 March 2018, <u>Biosecurity Advice 2018-06</u> (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 this draft report.

During preparation of this 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.

#### 1.2.5 Next steps

This draft report gives stakeholders the opportunity to comment and draw attention to any scientific, technical, or other gaps in the data, misinterpretations and errors.

The department will consider submissions received on this draft report and may consult informally with stakeholders. The department will then prepare a final report, taking into account stakeholder comments.

The department will publish the final report on its website along with a notice to stakeholders of the release. The department will also notify all registered stakeholders and the World Trade Organization Secretariat about the release of the final report. Publication of the 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.

## 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 (OIE), in its *Aquatic animal health code* (OIE Code), describes 'General obligations related to certification' in chapter 5.1 (OIE 2019e).

The OIE Code states in Article 5.1.2. that:

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

Article 5.1.2. further states that:

The international aquatic animal health certificate should not include measures against pathogenic agents or diseases that are not OIE 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. of the OIE 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.

#### 2.1 Risk review

Risk review is not defined or described in the OIE 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.

This draft risk review has drawn on the following sources of information (this list is not exhaustive):

• the OIE Code (OIE 2019b)

- the OIE Manual of diagnostic tests for aquatic animals (OIE 2019m)
- *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
- a review of relevant scientific literature
- expert opinion
- policies adopted by other countries for the importation of prawns.

Risk, defined by the OIE 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', 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 OIE 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 OIE 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 (OIE 2019f).

In accordance with the OIE Code, 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'.

Figure 1 shows the steps in the risk assessment process. <u>Chapter 4</u> further describes the method used to assess risk and the general considerations taken into account when undertaking this draft risk review.

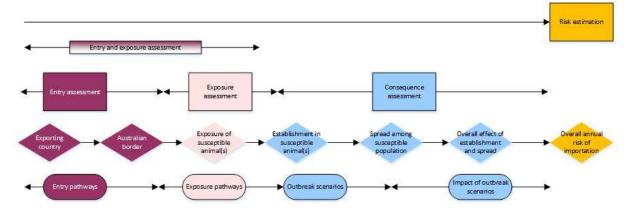


Figure 1 Steps in the risk assessment process

### 2.4 Review of risk management

The OIE Code (chapter 2.1) 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 OIE member's appropriate level of protection (ALOP).

Australia's ALOP has not changed since the Prawn IRA 2009 was published. Risk evaluation during this draft risk review was based on the conclusions drawn from the risk reviews 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 OIE 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 draft risk review, detailed consideration of numerous biosecurity measures for imported prawns was undertaken and documented (see chapter 5 <u>Options for biosecurity management of imported prawns</u>).

#### 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 is the ongoing process 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 OIE 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'.

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 draft risk review enables stakeholder feedback on draft conclusions and recommendations about Australia's biosecurity policies.

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 (OIE) as affecting prawns (and other species where relevant) (OIE 2020b)
- diseases identified in the *Generic import risk analysis report for prawns and prawn products 2009* (Prawn IRA 2009)
- other diseases identified as occurring in prawns.

The hazard identification process is described in section 2.2.

Table 1 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.

Many pathogenic agents are ubiquitous and may already be present in Australia. Others are opportunistic, not reported to be pathogenic, 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 <u>section 2.2</u>.

The pathogenic agents identified as hazards and retained for risk review are listed at the end of this chapter (see section 3.1 <u>Pathogenic agents retained for risk review</u>).

#### Table 1 Hazard identification and refinement

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (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 there have been no recent reports of this, which 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 (Crangon crangon nudivirus (CcNV))	Crangon crangon	No	Uncertain	No	Belgium United Kingdom	No	No: there is little evidence to indicate that bacilliform virus of <i>Crangon crangon</i> 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 & Stentiford 2017; Van Eynde et al. 2018)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason) to bacilliform virus of <i>Crangon</i>	Reference(s)
Baculoviral midgut gland necrosis virus (BMNV) and 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	<b>No:</b> BMNV is no longer of international significance in prawn health due to improved biosecurity and production practices. BMNV is no longer OIE- listed. BMNV is not included on the Australian <i>List of reportable</i> <i>diseases of aquatic animals</i> . 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 OIE- listed. BP is not included on the Australian <i>List of reportable</i> <i>diseases of aquatic animals</i> . 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 eggs and nauplii in	(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	OIE- 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 2020? (Yes/No: reason)	Reference(s)
							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	<b>No:</b> not considered to cause significant disease.	(Bateman & Stentiford 2017; Vogt 1996)
Beihai shrimp virus genotypes 1 -6, and 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 OIE. 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: <i>Macrobrachium</i> <i>rosenbergii</i> <i>Penaeus chinensis</i> <i>Penaeus japonicus</i> <i>Penaeus monodon</i> <i>Penaeus vannamei</i> Some finfish species: <i>Mugilogobius abei</i>	No	Yes	No	China Ecuador 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 <i>Penaeus vannamei</i> in culture. CMNV is not included on the Australian <i>List of reportable</i> <i>diseases of aquatic animals</i> . CMNV is included in the <i>List of Diseases in</i> <i>the Asia-Pacific</i> . This pathogenic agent complies with the criteria described in the <i>OIE Aquatic Animal Health Code</i> <i>Article 2.1.2 Hazard Identification</i>	(NACA, OIE-RRAP & FAO 2018; Wang et al. 2018; Zhang et al. 2014; Zhang et al. 2018; Zhang et al. 2017b)

Pathogenic agent (disease)	Susceptible species Paralichthys olivaceus	OIE- 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 2020? (Yes/No: reason) and will be retained for risk assessment.	Reference(s)
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 <i>Crangon crangon</i> <i>novel viruses</i> 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 <i>Crangon crangon</i> discovered the 15 novel viruses. <i>Crangon crangon novel viruses</i> have been detected in apparently healthy prawns. The department will continue to monitor the situation with respect to the <i>Crangon crangon novel</i> <i>viruses</i> .	(Van Eynde et al. 2020)
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 <i>M. rosenbergii</i> from China. There is little evidence to indicate that it would be associated with imported prawns and no evidence that prawn species other than <i>M. rosenbergii</i> are susceptible to infection. The department will continue to monitor the situation with respect to CHEV1.	(Dong et al. 2020b)
Decapod iridescent virus 1 (DIV1) (including: <i>Cherax quadricarinatus</i> iridovirus (CQIV)	Various penaeid and caridean species including: <i>Cherax</i> quadricarinatus	No	Yes	No	China Indian Ocean Taiwan	No: disease agent not identified in Prawn IRA 2009	<b>Yes:</b> the department notes that although there is limited information regarding DIV1, it is considered a serious emerging disease in aquaculture in China and appears to be spreading throughout the surroundings of	(Chen et al. 2019a; Chung 2020; Li, Xu & Yang 2017; NACA 2016; Qiu et al. 2017; Qiu et al. 2018a; Qiu et al. 2018b; Qiu et al.

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Shrimp hemocyte iridescent virus (SHIV))	Macrobrachium nipponense Macrobrachium rosenbergii Penaeus chinensis Penaeus japonicus Penaeus monodon Penaeus vannamei Various aquatic animals including: Cladocera (water flea) Procambarus clarkii (Exopalaemon carinicauda, Pachygrapsus crassipes and Eriocheir sinensis (experimental infection only))						farming areas in China – large volumes of imported prawns are sourced from areas that may be affected by DIV1. Recent reports indicate it may be present in Thailand. Complete genome sequencing has revealed that CQIV and SHIV are different strains or genotypes of the same virus. The genome of SHIV was shown to be 99% identical to the genome of CQIV. Recently, SHIV and CQIV were formally classified by the International Committee on Taxonomy of Viruses (ICTV) under the name Decapod iridescent virus 1 (DIV1) in the family Iridoviridae (ICTV 2018). DIV1 is included in the <i>List of diseases in the Asia-Pacific</i> , and proposed for inclusion as a disease notifiable to the OIE and in the Australia's National list of <i>reportable diseases of aquatic animals</i> . This pathogenic agent complies with the criteria described in the <i>OIE Aquatic Animal Health Code</i> <i>Article 2.1.2 Hazard Identification</i> and will be retained for risk assessment.	2019b; Ramsden & Smith 2018; Xu et al. 2016)
Decapod penstylhamaparvovirus 1, previously known as Decapod penstyldensovirus 1 and infectious hypodermal and	Various penaeid and caridean species including: Macrobrachium rosenbergii	Yes	Yes	Yes, multiple strains.	Africa Asia Central America Middle East	No	<b>No:</b> present in Australia and is not subject to control or eradication. IHHNV is listed by the OIE, is listed on the Australian <i>List of reportable</i>	(Bateman & Stentiford 2017; Lightner et al. 1994; Lu et al. 1991; OIE 20190; Owens et al. 1992;

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
haematopoietic necrosis virus (IHHNV) (includes multiple strains)	Penaeus californiensis Penaeus monodon Penaeus setiferus Penaeus stylirostris Penaeus vannamei				North America Pacific islands South America		diseases of aquatic animals and the List of diseases in the Asia-Pacific.	Rai et al. 2012; Srisala et al. 2020b)
Decapod hepanhamaparvovirus 1, previously known as Decapod hepandensovirus 1, Hepatopancreatic parvovirus (HPV) and <i>Penaeus monodon</i> densovirus (PmDNV) (includes multiple strains)	Various penaeid and caridean species including: Macrobrachium rosenbergii Penaeus californiensis Penaeus monodon Penaeus setiferus Penaeus setiferus Penaeus stylirostris Penaeus vannamei	No	Yes	Some strains	Africa Americas Asia Middle East	Yes	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(La Fauce, Elliman & Owens 2007; Srisala et al. 2020b; Walsh et al. 2017)
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 <i>Penaeus</i> <i>duorarum</i> discovered genome sequences of FdNV. FdNV has been only detected in healthy prawns. It is unknown if FdNV has the potential to cause disease. The department will continue to monitor the situation with respect to FdNV.	(Ng et al. 2013)
Hepatopancreas and digestive tract necrosis virus (HINV). (Glass post-larvae)	Penaeus vannamei	No	Uncertain	No	China	No: disease agent not identified in Prawn IRA 2009.	<b>No:</b> HINV is a recently described virus associated with mortalities in 5 to 10 days old <i>Peneaus vannamei</i> postlarvae in Chinese hatcheries. Disease by HINV has been referred to "glass post-	(Harkell 2020a, c)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
							larvae' as postlarvae become translucent before dying. There is little evidence to indicate that HINV would be associated with imported prawns as there is insufficient evidence that HINV cause disease in adult prawns. The department will continue to monitor the situation with respect to HINV.	
Infectious myonecrosis virus (IMNV)	Various penaeid species including: Penaeus esculentus Penaeus merguiensis Penaeus monodon Penaeus vannamei (Artemia franciscana, Penaeus stylirostris and Penaeus subtilis (experimental infection only))	Yes	Yes	No	Brazil Burma China India Indian Ocean Indonesia Malaysia Myanmar Republic of Korea	Yes	Yes: IMNV is OIE 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 OIE Aquatic Animal Health Code Article 2.1.2 Hazard Identification and will be retained for risk assessment.	(Coelho et al. 2009; Sahul Hameed et al. 2017; Tang et al. 2005)
Irido-like virus / Protrachypene precipua iridovirus	Protrachypene precipua	No	No	No	Ecuador	No	<b>No:</b> not considered to cause significant disease.	(Lightner & Redman 1993)
Laem Singh virus (LSNV) (including Wenzhou shrimp virus genotype 9 (WZSV9)) and an associated integrase- containing element (ICE)	Various penaeid species including: Penaeus dobsoni Penaeus merguiensis Penaeus monodon Penaeus vannamei	No	Yes	No	China India Indonesia Malaysia Philippines Sri Lanka Thailand	Yes (considered as MSGS): but there was insufficient information to conduct	Yes: MSGS is included on the Australian <i>List of reportable</i> <i>diseases of aquatic animals.</i> It has recently been determined that LSNV and WZSV9 are different isolates of the same virus species. Although there is limited information regarding LSNV and	(NACA 2016; Panphut et al. 2011; Poornima et al. 2012; Prakasha et al. 2007; Shi et al. 2016; Sittidilokratna et al. 2009b;

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
(as component causes of monodon slow growth syndrome (MSGS))					Vietnam	a risk assessment	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 <i>OIE Aquatic Animal Health Code</i> <i>Article 2.1.2 Hazard Identification</i> and will be retained for risk assessment.	Taengchaiyaphum et al. 2020)
Lymphoid organ vacuolization virus (LOVV)	Penaeus stylirostris Penaeus vannamei	No	No	No	Americas Hawaii	No	<b>No:</b> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens, De Beer & Smith 1991)
Macrobrachium nipponense reovirus (MnRV)	Macrobrachium nipponense	No	No	No	China	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> disease is currently restricted to <i>M. nipponense</i> from China. There is little evidence to indicate that it would be associated with imported prawns and no evidence that prawn species other than <i>M. nipponense</i> are susceptible to infection.	(NACA 2016; Zhang et al. 2016)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason) The department will continue to monitor the situation with respect	Reference(s)
Macrobrachium rosenbergii Golda virus (MrGV)	Macrobrachium rosenbergii	No	Uncertain	No	Bangladesh	No: disease agent not identified in Prawn IRA 2009	to MnRV. <b>No:</b> the disease is currently restricted to <i>M. rosenbergii</i> 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)
<i>Macrobrachium rosenbergii</i> 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 <i>M. rosenbergii</i> 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. The department will continue to monitor the situation with respect to MrTV.	(NACA 2016; Pan et al. 2016)
Monodon baculovirus (MBV) (Singly enveloped nuclear polyhedrosis virus from <i>Penaeus monodon</i> (PmSNPV)) (Includes plebejus baculovirus and bennettae baculovirus)	Various penaeid and caridean species, including: <i>Macrobrachium rosenbergii</i> <i>Penaeus indicus</i> <i>Penaeus merguiensis</i> <i>Penaeus monodon</i> <i>Penaeus penicillatus,</i>	No	No	Some strains	Americas Asia East Africa Madagascar Mediterranean (Italy) Middle East Indo Pacific region	No	<b>No:</b> some strains present in Australia, is not included on the Australian <i>List of reportable</i> <i>diseases of aquatic animals</i> 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)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Spherical baculovirus ( <i>Penaeus monodon</i> nudivirus (PmNV))	Penaeus semisulcatus Penaeus esculentus Penaeus kerathurus							
Mourilyan virus (MoV) (including 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 <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	Uncertain	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 & Stentiford 2017)
Penaeid haemocytic rod- shaped virus (PHRV)	Hybrid Penaeus esculentus × Penaeus monodon	No	No	Yes	Not reported	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens 1993a)
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)
<i>Penaeus vannamei</i> nodavirus (PvNV) (white tail disease-like muscle necrosis)	Penaeus vannamei (Penaeus monodon (experimental infection only))	No	Uncertain	No	Belize	No: disease agent not identified	<b>No:</b> not considered to cause significant disease.	(Tang et al. 2007b; Tang et al. 2011)

Pathogenic agent (disease)	Susceptible species	OIE- listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in Prawn IRA 2009? (Yes/No) in Prawn	Considered a hazard in 2020? (Yes/No: reason) The department will continue to	Reference(s)
						IRA 2009	monitor the situation with respect to PvNV.	
Reo-III and IV (including Reo-like virus and Palaemon B-cell reo-like virus) viruses (Penaeid shrimp Reo-like virus, Reo-like virus in <i>Penaeus vannamei</i> )	Various penaeid species	No	Uncertain	No	Americas Asia Europe	No	<b>No</b> : 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	<b>No:</b> there is little evidence to indicate that RPS would be associated with imported prawns as there is insufficient evidence that RPS is a pathogen of prawns.	(Biosecurity Australia 2009; Lightner 1996a; Lu et al. 1991)
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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens & McElnea 2000)
Taura syndrome virus (TSV)	Various penaeid species, including: Penaeus aztecus Penaeus ensis Penaeus indicus Penaeus monodon Penaeus setiferus Penaeus stylirostris	Yes	Yes	No	Americas China East Africa Hawaii Indonesia Malaysia Middle East Myanmar	Yes	Yes: TSV is OIE Listed and is associated with significant losses in prawn farming environment and is widespread throughout the world. TSV is included on the Australian <i>List of reportable</i> <i>diseases of aquatic animals</i> , and the <i>List of diseases in the Asia</i> - <i>Pacific</i> . This pathogenic agent complies with the criteria described in the	(Brock 1997b; Jimenez et al. 2000; Lightner 1996a)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
	Penaeus vannamei (Penaeus chinensis, Penaeus merguiensis and Macrobrachium rosenbergii (experimental infection only))				Republic of Korea Taiwan Thailand Vietnam		OIE Aquatic Animal Health Code Article 2.1.2 Hazard Identification and will be retained for risk assessment.	
Wenzhou Shrimp virus (WZSV) genotypes 2–8 and 10 (excluding: WZSV1 (refer Mourilyan virus) and WZSV9 (refer Laem Singh virus))	Various penaeid and caridean species including: Exopalaemon carinicauda Metapenaeus sp. Penaeus monodon Penaeus vannamei Solenocera crassicornis	No	No	Some strains present (WZSV1, 2 and 8)	China	No: disease agent not identified in Prawn IRA 2009	No: some strains present in Australia, is not included on the Australian <i>List of reportable</i> <i>diseases of aquatic animals</i> and is not subject to control or eradication. WZSV is not listed by the OIE. RNA-seq analysis of the transcriptome of prawn species discovered genome sequences of WZSV genotypes 1–10. WZSV have been detected in healthy prawns. The department will continue to monitor the situation with respect to exotic strains of WZSV.	(Huerlimann et al. 2018; Li et al. 2015; Shi et al. 2016; Taengchaiyaphum et al. 2020) (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 and eradication program.	Americas Asia East Africa Middle East	Yes	Yes: WSSV is OIE 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 on-going.	(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)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
							This pathogenic agent complies with the criteria described in the <i>OIE Aquatic Animal Health Code</i> <i>Article 2.1.2 Hazard Identification</i> and will be retained for risk assessment.	
White tail disease (WTD) ( <i>Macrobrachium</i> <i>rosenbergii</i> nodavirus ( <i>MrNV</i> ) and extra small virus (XSV) / <i>Macrobrachium</i> muscle virus (MMV))	Various penaeid and caridean species including: Macrobrachium rosenbergii Penaeus indicus Penaeus monodon Penaeus japonicus Penaeus vannamei	Yes	No	Yes	China Dominican Republic French West Indies India Indonesia Malaysia Myanmar Taiwan Thailand Vietnam	No	<b>No:</b> present in Australia and not subject to control or eradication. WTD is included on the Australian <i>List of reportable diseases of</i> <i>aquatic animals</i> and the <i>List of</i> <i>diseases in the Asia-Pacific</i> .	(Gangnonngiw et al. 2020; Kibenge & Godoy 2016; Murwantoko et al. 2016; Pillai & Bonami 2012; Ravi et al. 2009; Senapin et al. 2012; 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	Egypt Indonesia Malaysia Mexico Myanmar Philippines Sri Lanka Taiwan Thailand	Yes	Yes: YHV1 is OIE 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 <i>List of reportable</i> <i>diseases of aquatic animals,</i> and the <i>List of diseases in the Asia</i> - <i>Pacific.</i> This pathogenic agent complies with the criteria described in the <i>OIE Aquatic Animal Health Code</i> <i>Article 2.1.2 Hazard Identification</i> and will be retained for risk assessment.	(Chayaburakul et al. 2004; Flegel et al. 2004; Megahed, Cruz-Flores & Dhar 2018)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Yellow head virus genotypes 2–7 (YHV2– YHV7)	Various penaeid species	No	No	Some strains present (YHV2, YHV6 and YHV7)	China Egypt 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 OIE. Infection with gill associated virus (YHV2) is included on the Australian <i>List of reportable</i> <i>diseases of aquatic animals</i> . Infection with YHV2-YHV7 are not included in the <i>List of diseases in</i> <i>the Asia-Pacific</i> . YHV2, YHV6 and YHV7 are present in Australia. The susceptibility of <i>P. monodon</i> and <i>P. merguiensis</i> 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.	(Chen et al. 2018; Cowley et al. 2000; Cowley et al. 2015; Liu et al. 2014; Megahed, Cruz- Flores & Dhar 2018; Mohr et al. 2015; Munro, Callinan & Owens 2011; NACA, OIE- RRAP & FAO 2020a; OIE 2017a; Walker et al. 2001; Wijegoonawardane et al. 2008) (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 OIE listed. Infection with YHV8 is not included in the <i>List of diseases in</i> <i>the Asia-Pacific.</i> However, YHV8 is currently responsible for disease outbreaks in China. Large volumes of prawns are exported from China to Australia.	(Kim et al. 2020; Thitamadee et al. 2016; Zhu et al. 2016)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
							It is unknown if Australian prawns are susceptible to YHV8. This pathogenic agent complies with the criteria described in the <i>OIE Aquatic Animal Health Code</i> <i>Article 2.1.2 Hazard Identification</i> and will be retained for risk assessment.	
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 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 OIE listed or subject to control or eradication in Australia and their worldwide distribution is unknown. YHV9 and YHV10 are not included on the <i>List of diseases in the Asia-</i> <i>Pacific.</i> The department will continue to monitor the situation with respect to YHV9 and YHV10.	(Cowley et al. 2015; FRDC 2018) (FRDC Conference abstract, Moody et al 2019)
Chlamydia, mycoplasma, rickettsia, spiroplasma	_	-	-	-	_	-	-	-

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Chlamydia species	Various aquatic animals, including penaeid species	No	No	Yes	Ecuador	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Jimenez et al. 2001; Owens & Hall-Mendelin 1990)
<i>Mycoplasma</i> species	Various aquatic animals, including penaeid species	No	No	Yes	China	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Ghadersohi & Owens 1999)
Planctomycete bacteria	Penaeus monodon	No	No	Yes	Not reported	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Fuerst et al. 1997)
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 NHPB.	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 <i>"Candidatus</i> Hepatobacter penaei" (Necrotising hepatopancreatitis (NHP))	Various penaeid species including: Penaeus aztecus Penaeus duorarum Penaeus marginatus Penaeus merguiensis	Yes	Yes	No	Americas Malaysia Thailand United States of America Vietnam	Yes	<b>Yes:</b> <i>"Candidatus</i> Hepatobacter penaei" is OIE listed and associated with significant losses in prawn farming environment and is widespread throughout the world. <i>"Candidatus</i> Hepatobacter penaei" is included on the Australian <i>List of reportable</i>	(Nunan et al. 2013)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
(previously necrotising hepatopancreatitis bacterium (NHPB))	Penaeus setiferus Penaeus stylirostris Penaeus vannamei (Penaeus monodon (experimental infection only)) (Homarus americanus (pathogen specific PCR result but no active infection))						diseases of aquatic animals, and the List of diseases in the Asia- Pacific. This pathogenic agent complies with the criteria described in the OIE Aquatic Animal Health Code Article 2.1.2 Hazard Identification and will be retained for risk assessment.	
Rickettsia-like bacteria (RLB) associated with milky haemolymph disease (Milky haemolymph syndrome / milky haemolymph disease (MHD)) (Caused by 4 isolates of Rickettsia-like bacteria)	Carcinus maenas Panulirus species Penaeus monodon (Penaeus vannamei (experimental infection only))	No	Yes	No	East Africa France Madagascar Malaysia Mozambique Tanzania United Kingdom Vietnam	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 disease infection in cultured prawns, including one report of disease in cultured <i>Penaeus</i> <i>monodon</i> and <i>Penaues vannamei</i> 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.	(Australian Government Department of Agriculture Fisheries and Forestry 2012; Lightner et al. 2012b; Nunan et al. 2003a; Nunan et al. 2003b; Nunan et al. 2010)
Spiroplasma species, including Spiroplasma eriocheiris	Macrobrachium nipponensis Macrobrachium rosenbergii Penaeus vannamei Eriocheir sinensis	No	Yes	No	China Colombia	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> associated with disease in crabs and crayfish, but limited reports of <i>Spiroplasma</i> species infecting penaeid and <i>Macrobrachium</i> species. <i>Spiroplasma eriocheiris</i> infection is included in the <i>List of Diseases in</i> <i>the Asia-Pacific</i> .	(Liang et al. 2011; NACA, OIE-RRAP & FAO 2018; Nunan et al. 2005; Srisala et al. 2018; Wang et al. 2011)

Pathogenic agent (disease)	Susceptible species Procambarus clarkii	OIE- 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 2020? (Yes/No: reason) The department will continue to monitor the situation with respect	Reference(s)
Bacteria	-	-	-	-	-	_	to Spiroplasma species.	-
<i>Aerococcus viridans</i> var <i>homari</i> (gaffkemia)	Various aquatic animals, including: Penaeus aztecus Pandalus platyceros Homarus species Several crab species	No	Yes	No	Europe North America United Kingdom	No	<b>No:</b> there is little evidence to indicate that <i>Aerococcus viridans</i> var <i>homari</i> would be associated with imported prawns.	(Stebbing et al. 2012)
Aeromonas species	Various aquatic animals, including penaeid and caridean species such as: <i>Macrobrachium</i> <i>rosenbergii</i>	No	No	Some species present ( <i>Aeromonas</i> salmonicida subsp. salmonicida is exotic)	Widely distributed	No	No: present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication. (Note: <i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> is not associated with prawns)	(Pillai & Bonami 2012)
Aquatic epicommensal bacteria ( <i>Cytophaga</i> species <i>Flavobacterium</i> species <i>Leucothrix mucor</i> <i>Leucothrix</i> species <i>Thiothrix</i> species)	Various aquatic species, including penaeid and caridean species	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Lewis, Leong & Mock 1982)
<i>Bacillus cereus</i> (white patch disease)	Penaeus vannamei	No	No	Yes	Widely distributed	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Velmurugan et al. 2015)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Bacillus licheniformis	Penaeus vannamei	No	No	Yes	Colombia Presumed to be widely distributed.	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Galvez et al. 2016; Prada-Peñaranda et al. 2018)
Diplococcus species	Various aquatic animals, including penaeid species	No	No	Yes	Presumed to be widely distributed.	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens & Hall- Mendelin 1990)
Enterobacter cloacae	Various aquatic animals including penaeid and caridean species such as: <i>Macrobrachium</i> <i>rosenbergii</i> <i>Procambarus clarkii</i>	No	No	Yes	Widely distributed.	No	No: present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication. <i>E. cloacae</i> has been reported causing infections in China, where it has been associated with disease in farmed <i>P. clarkii</i> , and with slow growth and poor survival rates of <i>M. rosenbergii</i> in hatcheries. <i>E. cloacae</i> has also been reported causing mortality on the fish, <i>Mugil cephalus</i> , 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 <i>E. faecium</i>	Taiwan	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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:	No	No	Some species present	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of reportable diseases of aquatic</i>	(Escobedo-Bonilla 2016; Lightner 1985; Sheu et al.

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
	Macrobrachium rosenbergii Penaeus stylirostris						<i>animals</i> and is not subject to control or eradication.	2011; Uddin et al. 1998)
Flexibacter species	Various aquatic animals, including penaeid and caridean species	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No</b> : not considered to cause significant disease.	(Vogt 1997; Vogt & Strus 1998)
Lactococcus species. (Lactococcus 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Lalitha & Surendran 2004)
<i>Mycobacterium</i> species	Various aquatic animals, including penaeid and caridean species	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Brock, Nakagawa & Shimojo 1986; Lightner & Redman 1986)
Photobacterium species (Including:	Various aquatic animals including fish, mollusc,	No	No	Yes	Widely distributed	No: disease agent not identified	<b>No:</b> present in Australia, is not included on the Australian <i>List of reportable diseases of aquatic</i>	(Liu, Liu & Li 2016; Prayitno & Latchford 1995; Rivas, Lemos &

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Photobacterium damselae subsp. damselae and Ph. Phosphoreum)	penaeid and caridean species					in Prawn IRA 2009	<i>animals</i> and is not subject to control or eradication.	Osorio 2013; Singaravel et al. 2020; Vaseeharan et al. 2007)
Providencia species (Providencia rettgeri and P. alcalifaciens)	Various peneaid species including: <i>Penaeus vannamei</i> 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 <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication. <i>Providencia</i> species are not on the <i>Australian national notifiable</i> <i>diseases</i> or the <i>Non-national</i> <i>notifiable diseases in Australia's</i> <i>States and Territories</i> 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: <i>Macrobrachium</i> <i>rosenbergii</i>	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Pillai & Bonami 2012)
Red Body Disease Associated with: Aeromonas schubertii Proteous penneri Vibrio species (V. alginolyticus, V. harveyi, V. parahaemolyticus)	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, is not a listed disease 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)
Sherwanella algae	Various aquatic animals including: Penaeus vannamei	No	No	Yes	Widely distributed	No: disease agent not identified	<b>No:</b> present in Australia, is not included on the Australian <i>List of reportable diseases of aquatic</i>	(Cao et al. 2018a; Department of Health 2018b, a)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
	Known human pathogen					in Prawn IRA 2009	animals and is not subject to control or eradication. Sherwanella algae is not on the Australian national notifiable diseases or the Non-national notifiable diseases in Australia's States and Territories lists for human health.	
<i>Spirillum</i> species	Various penaeid species	No	No	Some species present	Widely distributed, including: Mexico	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Escobedo-Bonilla 2016)
<i>Staphylococcus</i> species	Macrobrachium rosenbergii	No	No	Some species present	Widely distributed, including: India	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Vijayan et al. 2005a)
<i>Streptococcus</i> species	Penaeus monodon Penaeus vannamei	No	No	Some species present	Widely distributed, including: Central America French Guiana Madagascar	No: disease agent not identified in Prawn IRA 2009	No: present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	Lightner 2009, Hasson et al 2009, (cited in (Lightner et al. 2012b))
Vibrio parahaemolyticus strains containing Pir toxins Vp <sub>AHPND</sub> (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 OIE but it did not satisfy the OIE case definition of AHPND as it was	Bangladesh China Costa Rica Egypt Malaysia Mexico Myanmar	No: disease agent not identified in Prawn IRA 2009	<b>Yes</b> : AHPND is listed by the OIE 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 <i>List of reportable</i> <i>diseases of aquatic animals</i> , and	(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 2016, 2017b,

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
				caused by a <i>Vibrio harveyi</i> clade and <i>Vp</i> <sub>AHPND</sub> was excluded.)	Peru Philippines Taiwan Thailand Vietnam		the List of diseases in the Asia- Pacific. AHPND means infection with strains of V. parahaemolyticus $(V_{PAHPND})$ that contain a ~70-kbp 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 $V_{PAHPND}$ 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 OIE Aquatic Animal Health Code Article 2.1.2 Hazard Identification and will be retained for risk assessment.	2019d; Tran et al. 2013b)
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 <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication. <i>Vibrio penaeicida</i> 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,

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
								Shimoyama & Momoyama 1985) (Jeremy Carson pers comm 2018)[Tasmania, Department Primary Industries Parks Water & Environment] 2018, pers. comm., 24 July)
Vibrio species (Including: species associated with luminous Vibriosis such as Vibrio harveyi. (Excluding: Vibrio parahaemolyticus strains containing Pir toxins VpAHPND)	Various aquatic animals including penaeid and caridean species	No	No	Some species present	Widely distributed	No	<b>No:</b> other than the <i>V</i> <sub>PAHPND</sub> (see above), <i>Vibrio</i> 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 <i>List of reportable</i> <i>diseases of aquatic animals</i> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Meyers 1990)
Aphanomyces species (Excluding: A. astaci (crayfish plague) A. invadans (epizootic ulcerative syndrome))	Various aquatic animals including: Daphnia magna Copepods Freshwater crayfish	No	No	Some species present	North America	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> not considered to cause significant disease.	(Sindermann 1976)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
	Macrobrachium rosenbergii							
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	<b>No:</b> not considered to cause significant disease.	(Karthikeyan, Gnanamoorthy & Gopalakrishnan 2014; Karthikeyan, Selvakumar & Gopalakrishnan 2015)
Atkinsiella dubia	Various marine crustaceans	No	No	Yes	Japan United States of America	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Lester & Paynter 1989)
Batrachochytrium dendrobatidis	Infects over 350 amphibian species	Yes	Yes	Yes (exception NT)	Widely distributed	No: disease agent not identified in Prawn IRA 2009	No: present in Australia. It is included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and subject to control. However, there is little evidence to indicate that <i>Batrachochytrium</i> <i>dendrobatidis</i> would be associated with imported prawns. <i>Batrachochytrium dendrobatidis</i> associated with prawns has been reported. However, it is questionable whether crustacean species are susceptible to <i>Batrachochytrium dendrobatidis</i> . 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	(AHC 2018; Department of Sustainability, Environment, Water, Population and Communities 2013; OIE 2019i; Paulraj et al. 2016; Pessier et al. 2017; Rowley, Alford & Skerratt 2006; Rowley et al. 2007)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
							dendrobatidis.	
Cladosporium species	Various aquatic animals including octopus and penaeid and caridean species such as: <i>Macrobrachium</i> <i>amazonicum</i>	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens & Hall- Mendelin 1990)
Enterocytozoon hepatopenaei (EHP) (Hepatopancreatic microsporidiosis (HPM), Enterosporidiosis)	Various penaeid species including: Penaeus japonicus Penaeus monodon Penaeus stylirostris Penaeus vannamei	No	Yes	No	Asia Venezuela	No: disease agent not identified in Prawn IRA 2009	<b>Yes:</b> EHP is associated with significant disease in Asia and is included on the Australian <i>List of reportable diseases of aquatic animals,</i> and the <i>List of diseases in the Asia-Pacific.</i> This pathogenic agent complies with the criteria described in the <i>OIE Aquatic Animal Health Code Article 2.1.2 Hazard Identification</i> 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 Fusarium solani (Burn spot disease, black gill disease, 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Humphrey 1995; Pillai & Bonami 2012)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Gilbertella persicaria	Penaeus monodon	No	No	No	Asia North and South America	No: disease agent not identified in Prawn IRA 2009	<b>No.:</b> not considered to cause significant disease. It has only been reported once in association with farmed <i>P. monodon</i> in India, and linked to unfavourable conditions including polluted water, high density and overfeeding.	(Karthikeyan & Gopalakrishnan 2014)
<i>Lagenidium</i> species (Larval mycosis)	Various aquatic animals including marine crustaceans, penaeid and caridean species such as: <i>Macrobrachium</i> <i>rosenbergii</i> <i>Penaeus monodon</i>	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 <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication. <i>Lagenidium</i> 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 (Leptolegnia marina = Leptolegniella marina = Salilagenidium marinum)	Crustaceans including: Penaeus monodon	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Humphrey 1995)
<i>Leptomitus</i> species	Various aquatic animals including freshwater and marine crustaceans	No	No	No	China France India United Kingdom United States of America	No	<b>No:</b> not considered to cause significant disease.	(Lightner 1993)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Microsporidian species (Including: Ameson species Agmasoma species Perezia species Pleistophora species Thelohania species Tuzetia species) (Excluding: Enterocytozoon hepatopenaei) (Cotton shrimp disease, milk shrimp disease)	Various decapod crustaceans.	No	No	Yes	Widely distributed	No	No: not considered to cause significant disease.	(Glazebrook, Owens & Campbell 1986; Owens & Glazebrook 1988)
Pythium species	Various aquatic animals including freshwater and marine crustaceans	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Humphrey 1995)
Sirolpidium species (= Haliphthoros species) (Larval mycosis, Brown spot disease)	Various mollusc and crustacean species including penaeids	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Lightner 1985; Meyers 1990)
Yeast	-	-	_	_	-	-	-	-

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Yeast species: (Including: Candida albicans Candida sake Debaryomyces hansenii Metschnikowia artemia Metschnikowia bicuspidate Metschnikowia kamienskii Metschnikowia pulcherrima Saccharomyces cerevisiae)	Various aquatic animal species including salmon and crustaceans, including: <i>Macrobrachium</i> <i>rosenbergii</i>	No	No	No	Taiwan	No	<b>No:</b> not considered to cause significant disease.	(Chen et al. 2003; Chen et al. 2007; Lu, Tang & Chen 1998; Pillai & Bonami 2012)
Protozoa	-	-	-	-	-	-	-	-
Apostome ciliates (Including: Ascophrys species Gymnodinoides species Synophrya species Hyalophysa species)	Penaeid species. (Other benthic decapods serve as hosts)	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Frischer et al. 2017; Landers et al. 2020; Lutz 2019; Owens et al. 1988; Paynter 1989)
<i>Bodo</i> -like flagellates <i>Chrysidella</i> species	Octopods and decapods	No	No	No	Widely distributed	No	<b>No:</b> not considered to cause significant disease.	(Humphrey 1995)
Gregarines (Including: <i>Cephalolobus</i> species <i>Nematopsis</i> species <i>Paraophioidina</i> species)	Various mollusc and penaeid species including: Penaeus esculentus Penaeus merguiensis	No	No	Yes	Widely distributed	No	<b>No:</b> not considered to cause significant disease.	(Jones 1998; Owens 1986)
Haplosporidan species (excluding Bonamia species)	Various penaeid species including: Penaeus duorarum	Only <i>Bonamia</i> ostreae and	No	Some Haplosporidan	Canada Cuba Nicaragua	No	<b>No:</b> some <i>Haplosporidan</i> species are present in Australia.	(DykovÁ, Lom & Fajer 1988; Jones 1998; Lightner 1996b; Nunan et al.

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
(Hepatopancreatic haplosporidiosis)	Penaeus esculentus Penaeus monodon Penaeus vannamei	Bonamia exitiosa		species are present	Indonesia Mexico Philippines Thailand United States of America		Haplosporidan species associated with Hepatopancreatic haplosporidiosis are not considered to be Bonamia spp. Only the Haplosporidan species Bonamia species, Bonamia ostreae and Bonamia exitiosa are included on the Australian List of reportable diseases of aquatic animals.	2007; Thitamadee et al. 2016; Utari et al. 2012)
Haplosporidian-like parasite (Red gill disease)	Macrobrachium nipponense	Only Bonamia ostreae and Bonamia exitiosa	Unknown	Some Haplosporidan species are present	China	No: disease agent not identified in Prawn IRA 2009	No: Red gill disease is thought to be associated with haplosporidian-like parasites found in the gills of <i>M. nipponense</i> . Haplosporidian-like parasite associated with red gill disease has not been considered to be <i>Bonamia</i> spp and it is considered a potentially new haplosporidian pathogen due to its unique spore ornamentation. Some <i>Haplosporidan</i> species are present in Australia. Only the <i>Haplosporidan</i> species <i>Bonamia</i> species, <i>Bonamia ostreae</i> and <i>Bonamia exitiosa</i> are included on the <i>Australian List of reportable</i> <i>diseases of aquatic animals</i> . The department will continue to monitor the situation with respect to red gill disease.	(Ding et al. 2019)
Hematodinium species	Various crab species, some penaeid and caridean species including: <i>Exopalaemon</i> <i>carinicauda</i>	No	No	Some members of the genus present.	Widely distributed	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Gornik, Cranenburgh & Waller 2013; Hudson & Shields 1994; Wang et al.

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
	Penaeus monodon							2017b; Xu et al. 2010)
Hematodinium-like species (Spot prawn parasite (SPP))	Various caridean species including: Pandalus borealis Pandalus platyceros	No	No	No	Canada United States of America	No	<b>No:</b> 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	<b>No:</b> not considered to cause significant disease.	(Humphrey 1995; Lightner 1996b)
Paramoeba-like sp	Penaeus vannamei Various crustacean, echinoderm and finfish species	No	No	Yes	Widely distributed	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Han 2019)
Parauronema species	Various marine molluscs and crustaceans including: Penaeus aztecus	No	No	No	United States of America	No	<b>No:</b> not considered to cause significant disease.	(Bower, McGladdery & Price 1994; Couch 1978)
Peritrichous and loricate ciliates (Including: <i>Cothurnia</i> species <i>Epistylis</i> species <i>Lagenophrys</i> species <i>Rhabdostyla</i> species <i>Stylohedra</i> species <i>Vorticella</i> species <i>Zoothamnium</i> species)	Marine and freshwater crustaceans including: <i>Callinectes sapidus</i> <i>Cherax rotundus</i> <i>setosus</i> <i>Jasus edwardsii</i> <i>Macrobrachium</i> species <i>Penaeid</i> species	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Humphrey 1995; Paynter 1989)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Scuticociliates (Metanophrys sinensis)	Macrobrachium rosenbergii	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Piazzon, Leiro, & Lamas, 2014) cited in (Sahoo et al. 2018)
Suctorian ciliates (Including: <i>Acineta</i> species <i>Ephalota</i> species <i>Terebrospira</i> species)	Marine and freshwater crustacea including: <i>Palaemon</i> species <i>Palaemonetes</i> species <i>Penaeus</i> species	No	No	No	Widely distributed	No	<b>No:</b> not considered to cause significant disease.	(Humphrey 1995)
Thalassomyces species	Decapods	No	No	No	Widely distributed	No	<b>No:</b> not considered to cause significant disease.	(Humphrey 1995)
Metazoa	-	-	_	-	-	-	-	-
Anisarthus species	Caridean species including: <i>Athanas</i> species	No	No	No	Japan	No	No: not considered to cause significant disease.	(Nakashima 1995)
Anisorbione species	Various penaeid species	No	No	Yes	Philippines	No	No: present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Paynter 1989)
Ascarophis species	Homarus americanus Penaeus merguiensis	No	No	Yes	Barents Sea (Northern Scandinavia Russia) United States of America	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens 1987)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Austogathona species	Macrobrachium species	No	No	Yes	Not reported	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	Brock 1983 cited in (Paynter 1986)
Bopyrella species, Bopyrinella albida	Various caridean and mollusc species	No	No	Yes	Japan	No	<b>No:</b> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens 1993a)
Diceratocephala species	Decapod crustaceans	No	No	Yes	New Guinea	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Edgerton et al. 2002)
<i>Epipenaeon</i> species	Various penaeid species, including: Penaeus semisulcatus	No	No	Yes	India Indo-West Pacific Israel Persian Gulf Red Sea South Africa	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens & Glazebrook 1985)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
					Suez Canal Turkey			
Eutetrarhynchus species	Various penaeid species	No	No	Yes	India Tunisia	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> not considered to cause significant disease.	(Nakashima 1995)
Ionella maculate	Callianassa species	No	No	No	New Caledonia Tropical Indo- Pacific	No	<b>No:</b> not considered to cause significant disease.	(Markham 1994)
Kronborgia caridicola	Various crustaceans including caridean species and ampeliscid amphipod	No	No	No	Greenland	No	<b>No:</b> not considered to cause significant disease.	(Meyers 1990)
<i>Metaphrixus</i> species	Various caridean species including: <i>Palaemonella</i> species	No	No	Yes	New Caledonia Singapore United States of America	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> not considered to cause significant disease.	(Meyers 1990; Owens 1987)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Nectonema species	Various decapod crustaceans including caridean and brachyuran species	No	No	No	Canada New Zealand Norway United States of America	No	<b>No:</b> not considered to cause significant disease.	(Meyers 1990)
Opecoeloides fimbriatis	Various marine crustaceans including penaeid species	No	No	No	United States of America	No	<b>No:</b> not considered to cause significant disease.	(Owens 1987)
<i>Opecoeloides</i> species	Various aquatic animals including finfish species, and penaeid and caridean species such as: <i>Macrobrachium</i> <i>australiensis</i> <i>Penaeus vannamei</i>	No	No	Yes	Mexican Pacific United States of America	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Cribb 1987; Owens 1987)
Orbione halipori	Various penaeid species	No	No	Yes	Indo-West Pacific	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Humphrey 1995)
Parachristianella dimegacantha	Various penaeid species	No	No	No	Gulf of Mexico	No	<b>No:</b> not considered to cause significant disease.	(Owens 1987)
Parachristianella monomegacantha	Various penaeid species and bivalve mollusc	No	No	Yes	Widely distributed	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens 1987)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Parapenaeon species	Various penaeid species	No	No	Yes	Indo-West Pacific Pakistan	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens & Glazebrook 1985)
Parapenaeonella lamellate	Various penaeid species	No	No	Yes	China Hong Kong India	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Humphrey 1995; Owens 1987; Owens & Glazebrook 1985)
Probopyrus species	Various caridean species including: <i>Palaemonoidea</i> species	No	No	Yes	Atlantic seaboard India Malaysia United States of America	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens 1987)
Pseudophyllodistomum johnstoni	Freshwater finfish species and caridean species including: <i>Macrobrachium</i> species	No	No	Yes	Japan	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Humphrey 1995)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
Rhadinorhynchids	Penaeus merguiensis Various marine finfish Cephalopodo: Ommastrephes bartrami	No	No	Yes	Fiji Japan Northwest Pacific Ocean Peru Vietnam	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens 1987)
Sacculina species	Various brachyuran species	No	No	Yes	Ireland Japan Mediterranean Coast Sweden Taiwan Turkey United Kingdom	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> not considered to cause significant disease.	(Meyers 1990; Nagler et al. 2017)
Temnocephala carpentariae	Macrobrachium species Pomacea canaliculata	No	No	Yes	Argentina	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Damborenea 1996; Humphrey 1995)
Thynnascaris species (= Contracaecum species = Hysterothylacium)	Various penaeid and caridean species	No	No	Yes	Barents Sea (Northern Scandinavia Russia) Canada	No	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> and is not subject to control or eradication.	(Owens 1987)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
					El Salvador Mexican Pacific United States of America			
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	<b>No:</b> 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 a 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 in Prawn IRA 2009	No: not considered to cause significant disease and whilst the aetiology is unknown, it is likely associated with nutritional deficiency. The department will continue to monitor the situation with respect	(Kumar, Rao & Rao 2004; Pillai & Bonami 2012; Pillai et al. 2005)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason)	Reference(s)
							to appendage deformity syndrome.	
Blue body syndrome	Penaeus vannamei	No	No	No	China	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> 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) / 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 <i>Rickettsiales</i> and <i>Tenacibaculum</i> 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 <i>Rickettsiales</i> and <i>Tenacibaculum</i> . 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) / moult death syndrome (MDS) / metamorphosis moult mortality syndrome	Macrobrachium rosenbergii	No	No	No	Asia	No: disease agent not identified in Prawn IRA 2009	<b>No:</b> not considered to cause significant disease and whilst the aetiology is unknown, it is likely associated with nutritional deficiency.	(FAO 2020; Pillai & Bonami 2012; Soundarapandian & Varadharajan 2013)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason) The department will continue to	Reference(s)
							monitor the situation with respect to EED/MDS.	
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	<b>No:</b> 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	<b>No:</b> present in Australia, is not included on the Australian <i>List of</i> <i>reportable diseases of aquatic</i> <i>animals</i> 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	<b>No:</b> whilst previously associated with white faeces disease, recent 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 in Prawn IRA 2009	<b>No:</b> not considered to cause significant disease. Aetiology is unknown.	(Li et al. 2016)

Pathogenic agent (disease)	Susceptible species	OIE- 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 2020? (Yes/No: reason) The department will continue to monitor the situation with respect to secret death disease.	Reference(s)
White faeces syndrome (also known as white faeces disease / septic hepatopancreatic necrosis (SHPN))	Penaeus monodon Penaeus vannamei	No	No	Some species present	China India Indonesia Latin America Malaysia Thailand Vietnam	No: disease agent not identified in Prawn IRA 2009	No: aetiology is unknown, but is thought to be associated with a wide range of <i>Vibrio</i> species and gregarine-like bodies, some of which 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. May also be associated with infection with EHP (refer <i>Enterocytozoan hepatopenaei</i> ) and AHPND (refer <i>Vibrio</i> <i>parahaemolyticus</i> strains containing Pir toxins <i>Vp</i> AHPND). The department will continue to monitor the situation with respect to white faeces syndrome.	(Aranguren et al. 2019; Huang et al. 2020b; Limsuwan 2010; Mastan 2015; Sriurairatana et al. 2014; Tang et al. 2016; Towers 2016; Tran et al. 2017; Wang et al. 2020a)

# 3.1 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 General considerations and risk assessment process

This chapter provides details on the general considerations taken into account by the department when undertaking this draft risk review. Where relevant, explanation is provided for changes in assumptions or conclusions between this draft risk review and the *Generic import risk analysis report for prawns and prawn products 2009* (Prawn IRA 2009) (Biosecurity Australia 2009). When the general considerations are discussed in relation to each step in the risk assessment process, a detailed explanation of the risk assessment methodology is also provided at that point.

# 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 draft 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 2.

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

Table 2 Nomenclature for qualitative likelihoods

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. Evaluation of such factors formed the basis of the overall likelihood assigned to the entry and exposure assessments, and the likelihood of establishment and spread.

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 to consider when estimating the expected volume of trade. Table 3 shows the volume of prawns and prawn products exported to Australia during the 2019 calendar year (1 January 2019 to 31 December 2019).

Table 3 Volume of prawns and prawn products imported into Australia during 2019 calendar year

Commodity	Volume (kg)
Cooked <sup>a</sup>	12,288,667
Uncooked (raw) prawns <sup>a</sup>	8,443,005
Breaded, battered and crumbed <sup>a</sup>	2,388,013
Dumpling and dim sum-type product <sup>a</sup>	761,068
Other prawn products <sup>b</sup>	1,037,040
Total	24,917,793

<sup>a</sup> Imported according to the conditions outlined in Attachment A <u>Biosecurity Advice 2018-15</u> (Department of Agriculture and Water Resources 2018b). <sup>b</sup> Other prawn products refers to all shelf stable prawn products including dried shrimp, prawn crackers and shelf-stable prawn paste. An import permit is not required for these goods. Import conditions for these goods are available on the <u>Australian Biosecurity Import Conditions</u> website. These goods are outside the scope of this draft risk review.

# 4.2 Entry assessment

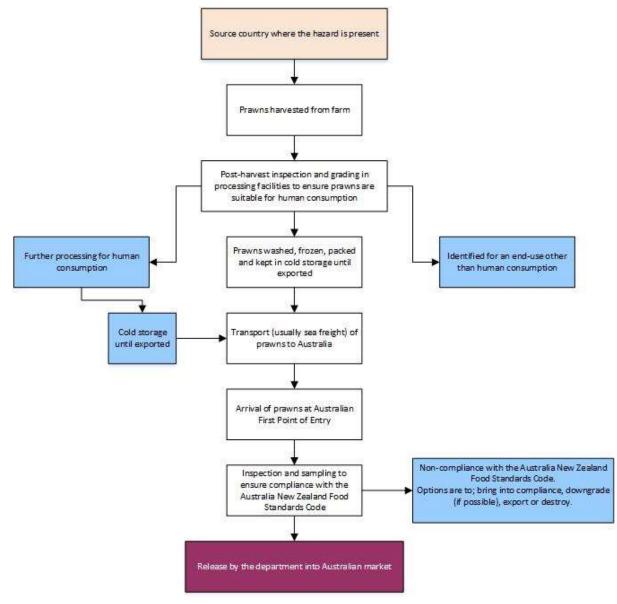
The World Organisation for Animal Health (OIE) *Aquatic animal health code* (OIE Code) (OIE 2019f) describes the entry assessment as:

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 draft 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 2 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).

#### Figure 2 Entry pathway for imported prawns into Australia



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 draft 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 <u>section 5.1.1</u>).

# 4.2.1 Key factors considered in entry assessment

## Biological characteristics of the hazard in harvested prawns

Described below are biological factors of the hazards that are considered during the entry assessments.

## 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 2019k). Other pathogenic agents are generally more restricted in host range, such as infectious myonecrosis virus (IMNV), which only infects certain *Penaeus* species (OIE 2019i).

# 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 2019a). 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 *P. 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  $(2.00 \times 10^{10} \text{ WSSV copies/g tissue})$  and 51% in the whole tail (shell and meat)  $(1.53 \times 10^{10} \text{ WSSV copies/g tissue})$  (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 <u>Australia New Zealand Food Standards Code</u> (FSC), developed by Food Standards Australia New Zealand (FSANZ), and the <u>Country of Origin Food Labelling Information Standard 2016</u>. The <u>Imported Food Control Act 1992</u> and its subordinate legislation (the <u>Imported Food Control Order 2019</u> and <u>Imported Food Control Regulations 2019</u>) 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 reduced in April 2020. The department has received risk advice from FSANZ that imported cooked prawns will remain a risk food only for *Vibrio cholera* testing (although still subject to surveillance tests for nitrofurans and fluoroquinolones). The products are tested as shown in Table 4.

Product	Risk tests	Surveillance tests
Uncooked prawns	na	Nitrofurans <sup>a</sup>
		Fluoroquinonolones <sup>a</sup>
		Label and visual assessment
Cooked prawns	Vibrio cholerae	Nitrofurans <sup>a</sup>
	Standard Plate Count	Fluoroquinolones <sup>a</sup>
	Label and visual assessment	

Table 4 The imported food inspection scheme requirements for imported prawns

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 <u>website</u>.

#### 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 <u>Postharvest inspection and grading</u> in section 4.2.1). 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 strains of *Vibrio parahaemolyticus* are sensitive to refrigeration (OIE 2019a). 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.

# 4.2.2 Conclusion

The amount of a pathogenic agent in prawns exported to Australia will depend on many factors, including the species of prawn, the pathogenic agent, the production system, and the stability of the pathogenic agent during and post-processing.

Inspection and grading procedures typically focus on human health concerns and the aesthetics, or acceptability, of the commodity to the consumer. The Prawn IRA 2009 concluded that prawns with gross abnormal signs such as visible lesions and blemishes, including those resulting from infection, may be rejected during inspection and grading prior to export to Australia. The Prawn IRA 2009 also noted that prawns that are free of external clinical signs (for example, subclinical infection) or that have subtle lesions are likely to pass post-harvest inspection. This conclusion is still valid.

Overall, it is considered that post-harvest inspection and grading procedures represent imperfect tests for addressing any biosecurity risks and at best may be useful for removing grossly abnormal prawns from the supply chain. The following risk assessments considered available scientific data on the likelihood that infected animals would show clinical signs or be infectious without showing clinical signs, and therefore the likelihood that infected animals would be identified by their appearance prior to export to Australia.

The Prawn IRA 2009 concluded that on-arrival inspection in Australia for the IFIS may reduce the entry risk if prawns are grossly abnormal and appear to be unsuitable for human consumption. It is now considered that given the low inspection rate for uncooked and cooked (once a compliance history is established) prawns as part of the IFIS, and the reduction of testing applied to cooked prawns, it is unlikely to reduce significantly the entry of imported prawns affected by hazards.

Washing will likely reduce the amount 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 viability of the pathogenic agents will be hazard specific.

# 4.2.3 Estimation of entry assessment

The entry assessments considered the above factors affecting the likelihood of each hazard entering Australia in imported prawns and estimated an annual likelihood of entry. The entry assessment used the qualitative likelihood descriptors described in Table 2.

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). All estimates of the likelihood of exposure assume the hazard is present in the imported prawns at the time of arrival in Australia.

Estimation of the likelihood of an exposure group encountering a hazard takes into account for each major exposure pathway the following factors (from entry into Australia, through storage, transport, end-use and any associated waste disposal):

- 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 draft 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.

These three exposure groups remain unchanged from the Prawn IRA 2009.

It is noted that covert mortality nodavirus (CMNV) has been reported to infect not only crustaceans, but also three finfish species (Wang et al. 2018; Zhang et al. 2018). Should information become available that demonstrates non-crustacean species native to Australia are susceptible to CMNV, the definition of the exposure groups may change with respect to the CMNV risk assessment.

# 4.3.2 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 draft 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 <u>Use of imported prawns as feed for crustacean broodstock and crustaceans in research facilities and public aquaria</u> (see section 4.3.3). 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).

This draft risk review identified the following (major) pathways as substantially contributing to the total risk:

- 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 <u>Appendix 1</u>).

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 <u>Appendix 1</u>) but were not considered further when conducting the risk assessments for this draft 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 <u>Biosecurity Act 2015</u> along with its subordinate legislation the <u>Biosecurity (Ballast Water and Sediment) Determination 2017</u> and the <u>Biosecurity (Ballast Water Same Risk Area) Instrument 2017</u>.

# Figure 3 depicts the most likely (major and minor) pathways by which the three exposure groups could be exposed to imported prawns in Australia.

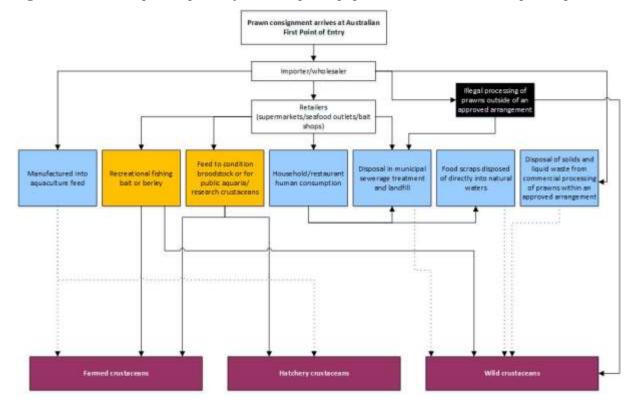


Figure 3 Potential exposure pathways of susceptible populations in Australia to imported prawns

Major exposure pathways that substantially contribute to total risk are orange boxes and indicated by a full line (see <u>Major</u> <u>exposure pathways</u> in section 4.3.3). Minor exposure pathways are light blue boxes and indicated with a dotted line (see <u>Minor exposure pathways</u> in Appendix 1). The black box is associated with an illegal pathway and is not within the scope of this draft risk review.

# 4.3.3 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 3):

- 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 <u>section 4.3.2</u> and <u>Appendix 1</u>) 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 draft 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 draft 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 1123 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 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 are a preferred option for recreational fishers with 62.6% of recreational fishers reporting use of prawns in a 12 month period (Kewagama Research 2002). In 2017, it was reported that plastic lures, uncooked prawns and caught fish were the most common baits used by respondents (Kantar Public 2017).

#### 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).

More recent 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). 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). In 2017, the Kantar Public survey found that 23% of fishers reported using uncooked prawns purchased from a supermarket and 6% reported using leftover cooked prawns from a meal as fishing bait. A follow-up survey was conducted in 2019 and the results were not statistically different (Kantar Public 2019). During the department's investigations following the WSD outbreak in 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 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 2007 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.

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) (Kewagama Research 2007).

During investigations into the WSD outbreak in 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).

# 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 is 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 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.

# 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).

More recently, 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 surveys discussed in this report show a steady increase since 2002, and more recently a relatively stable driver, of 'convenience' as the primary reason why prawns 'sold as seafood' are used as bait or berley. Convenience is likely a key factor in determining the form of prawn purchased from a supermarket and used as bait or berley. That is, if uncooked whole prawns were not available in the seafood retailer or their price was prohibitive, uncooked prawns with head and shell-removed may be preferred to no prawns or going to a bait shop.

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 and price.

#### 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. <u>Appendix 2</u> 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.

#### Summary

The use of imported prawns intended for human consumption as bait or berley is an exposure risk primarily for wild crustaceans. However, the Prawn IRA 2009 also identified the potential for hazards to be introduced directly into the environment of farmed crustaceans via the use of

imported prawns as bait in prawn farm inlet channels. This practice could result in infected prawn tissues entering ponds through intake water. It was considered a potentially significant exposure pathway (especially for WSSV) in the Prawn IRA 2009. This pathway still represents a direct and therefore potentially significant exposure pathway for farmed crustaceans. However, completion of this exposure pathway is less likely than wild crustaceans being directly exposed to imported prawns used as bait or berley.

The likelihood of susceptible wild crustaceans encountering imported prawns used as bait or berley by recreational fishers will depend on a number of factors (see section 4.3.5 <u>Contact</u> <u>between susceptible host animals and the prawns (or associated wastes</u>)). Crustaceans, including prawn species, are widely distributed in fresh and marine waters in Australia. Many of the waters in which recreational fishing occurs would be home to multiple crustacean species. Competition with non-susceptible aquatic species would reduce the likelihood of susceptible crustaceans consuming prawns potentially containing infectious organisms. Finfish species will consume a high proportion of prawns introduced into the aquatic environments as bait or berley. However, the nature of many popular fishing spots is such that fishing bait, which may include imported prawns, often enters a circumscribed body of water, such as an estuary or mangrove system. This would increase the probability of susceptible species present encountering imported product, compared to bait-use in open water.

The total volume (imported and Australian origin) of prawns purchased from supermarkets and used for bait or berley is significant, the volume entering the aquatic environment is substantial and it is a frequent and repeated activity. Currently, the department does not consider it is possible to prevent recreational fishers using imported prawns as bait or berley. This is because of a number of factors such as limitations on the practicality, enforceability and presence of legislation in states and territories to prohibit this activity, no point of sale requirements for labelling, educational campaigns are not nationally effective or implemented, and most importantly; convenience is the main driver for purchasing supermarket prawns as bait or berley. Further, the department considers that the relative volume of Australian origin and imported prawns purchased from supermarkets and used as bait will fluctuate depending upon availability in the supermarket, cost, quality and suitability (product form) for bait or berley use. Therefore, this draft risk review 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.

# 2) Use of imported prawns as feed for crustacean broodstock and crustaceans in research facilities and public aquaria

Uncooked prawns are 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 (Chimsung 2014; 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 polychaete worms 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 fed imported prawns caused a national emergency animal disease response due to the suspected establishment of WSSV in Australia. In that instance, the prawns, imported for human consumption, were considered to be 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 recently been advised by the jurisdictions and industry that in Australia it is highly unlikely that prawn aquaculture farms or hatcheries would condition their broodstock with prawns intended for human consumption, especially since the WSD outbreak in 2016. Although, the department is aware of seafood (non-crustacean based) imported for human consumption, being used as feed for hatchery animals in Australia as recently as 2017.

Taking the above information into account it is considered that the use of imported prawns as feed in farms or hatcheries is an unlikely exposure pathway. However, if this were to occur it would be a direct and potentially significant exposure pathway with a high likelihood of completion. Therefore, this exposure pathway is still considered a major exposure pathway.

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 <u>Appendix 2</u>) 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.

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.

# 4.3.4 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 draft 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  $2.62 \times 10^6$  genome copies/µL 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 2019a; 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 highrisk 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).

# 4.3.5 Contact between susceptible host animals and imported prawns (or associated wastes)

In Australia, the main aquaculture species are *P. monodon, Penaeus merguiensis* (Australian Prawn Farmers Association 2019) and *Melicertus plebejus* (State of Queensland 2018). The main target species for fisheries are *P. merguiensis, Penaeus indicus, Melicertus latisulcatus, Melicertus longistylus, M. plebejus, P. monodon, Penaeus esculentus and Penaeus semisulcatus* (Mobsby & Curtotti 2020).

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.

## 4.3.6 Conclusion

The minor exposure pathways (refer section 4.3.2 and <u>Appendix 1</u>) 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 draft risk review.

The Prawn IRA 2009 concluded that the regular introduction of prawn material into the aquatic environment through use as bait or berley presented a significant pathway by which wild crustaceans could be exposed to potentially infected imported prawns. This conclusion is still valid. This draft risk review considers that the use of imported prawns as bait or berley represents the most likely exposure pathway for wild crustaceans to hazards identified in this draft risk review.

It is viewed that the total volume of prawns purchased from supermarkets (which could be of Australian origin or imported) and used for bait or berley is significant and a frequent activity. At this time, it is not considered possible to prevent recreational fishers from using imported prawns as bait or berley. Therefore, this draft 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 unsuitable (for example, cooked product).

The Prawn IRA 2009 noted that if appropriate inlet filtration systems were not in place on prawn aquaculture farms, imported prawns used as bait in and around farm inlet and outlet channels may be a direct pathway for exposure of farmed prawns. Whilst this is now viewed to

be less likely than reported in the Prawn IRA 2009 (due to improvements in entry-level biosecurity on farms in some regions) it represents a direct pathway with a high likelihood of completion, and is therefore a potentially significant exposure pathway for farmed crustaceans. For that reason, it is considered further in this draft risk review. The Prawn IRA 2009 considered it unlikely that imported prawn tissue would be carried to aquaculture ponds by natural means such as wild birds. In this draft risk review, this potential exposure pathway is considered when determining the likelihood of farmed crustaceans being exposed to imported prawns used as bait or berley.

In this draft risk review, crustaceans kept in hatcheries, research institutions and public aquaria were considered overall to be the least likely to be deliberately or inadvertently exposed to imported prawns used as bait or berley 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 is considered less likely (however a direct and therefore significant pathway if it were to occur) than the use as feed for crustaceans in research institutions and public aquaria.

The Prawn IRA 2009 identified the potential for hazards to be introduced directly into the environment of farmed crustaceans via feeding of whole uncooked prawns to broodstock kept in maturation ponds on farms. This is no longer considered as likely as reported in 2009. However, it represents a direct and therefore potentially significant exposure pathway for farmed crustaceans.

Overall, of the three exposure groups and the two major exposure pathways, the most likely scenario that a susceptible population in Australia could be exposed to imported prawns is considered to be wild crustaceans exposed to imported prawns used as bait or berley by recreational fishers.

The department notes that since the 2002 and 2007 surveys, recreational fishers' behaviours will have likely changed and that more current data are required. The Kantar Public surveys in 2017 and 2019 have provided good information; however, they are Queensland focused and do not provide population-based outputs. The Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) is conducting the National social and economic recreational fishing survey (in conjunction with the University of Canberra), a statistically robust and repeatable online survey that is targeting 4,000-6,000 respondents across Australia. As part of this survey, data will also be gathered on the current use of prawns as bait or berley by recreational fishers. The outputs of the National social and economic recreational fishing survey will be validated against probability-based concurrent state-wide surveys in the Northern Territory, Queensland and New South Wales and recent surveys in Tasmania and Western Australia. The surveys will provide current scientifically robust data about the use of prawns intended for human consumption as bait or berley, including the use of cooked prawns, uncooked prawns which have had the head and shell removed and highly processed prawns (value-added products). Data from the *National social and economic recreational fishing survey* will be included in this report when available and any assumptions or outcomes will be adjusted should the data demonstrate it is required.

# 4.3.7 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 2).

# 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 4.2.3 <u>Estimation of entry assessment</u>) and the corresponding partial likelihood of exposure (PLE) (see section 4.3.7 <u>Estimation of partial likelihood of exposure</u>) using the matrix for combining descriptive likelihoods (Figure 4).

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		

Figure 4 Matrix for determining the partial annual likelihood of entry and exposure

# 4.5 **Consequence assessment**

According to the OIE Code, a consequence assessment should describe the potential consequences of a given exposure and estimate the probability of them occurring (OIE 2019f).

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

- 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

The Prawn IRA 2009 considered the following to be the 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 draft risk review, the following 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 2014)). 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.

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

The following factors were considered relevant when estimating the 'partial likelihood of establishment and spread' (PLES):

- 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 draft risk review, data are not available describing a 'true minimum infectious dose' (refer <u>Tissue tropism and infectious dose</u> in section 4.2.1 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 water borne 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 may 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 decreases 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, 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 restriction 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 postlarvae 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 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 restriction 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.

In Australia, the main aquaculture species are *P. monodon*, *P. merguiensis* (Australian Prawn Farmers Association 2019) and *Melicertus plebejus* (State of Queensland 2018). The main target species for prawn fisheries are (Mobsby & Curtotti 2020):

- black tiger prawn (*Penaeus monodon*)
- brown tiger prawn (*Penaeus esculentus*)
- grooved tiger prawn (Penaeus semisulcatus)
- banana prawn (*Penaeus merguiensis*)
- red-legged banana prawn (Penaeus indicus)
- Kuruma prawn (*Penaeus japonicus*)
- blue Endeavour prawn (Metapenaeus endeavouri)
- red Endeavour prawn (*Metapenaeus ensis*)
- school prawn (Metapenaeus macleayi)
- greasyback prawn (*Metapenaeus bennettae*)
- western king prawn (Penaeus latisulcatus)
- eastern king prawn (*Melicertus plebejus*)
- red-spot king prawn (*Penaeus longistylus*)

- coral prawn (*Metapenaeopsis crassissima*)
- giant freshwater prawn (Macrobrachium rosenbergii)
- freshwater prawn (Macrobrachium australiense).

Some of the hazards in this draft 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 2019i). 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 2019k) 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.

#### 4.5.3 Conclusion

The likelihood that a hazard will establish and spread, is influenced by a number of factors including the likelihood that animals would be exposed to an infectious dose, transmission pathways, the presence and density of susceptible animals and the likelihood that infected animals would be removed by non-susceptible species.

The Prawn IRA 2009 considered that disease establishment and spread was more likely in the case of farmed and hatchery crustacean populations, than in wild crustacean populations. The department considers this conclusion remains valid. This is because of the high density of susceptible host 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 compared to wild crustacean populations.

In the wild, consumption of diseased prawns by non-susceptible animals (such as finfish or birds), rather than susceptible host animals, may reduce the likelihood of establishment and spread. However, factors such as an environment conducive to increased protection from non-susceptible predators would increase the risks of establishment and spread in a wild population.

Indirect exposure routes are considered 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 (initially exposed to and infected by, an imported prawn) 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 less likely due to the closed systems, stronger biosecurity procedures and water treatment in place for these facilities.

The Prawn IRA 2009 considered that the dilution of effluent water from aquaculture ponds would be expected to reduce the amount of a hazard present, and therefore reduce the likelihood that this effluent would cause infection in populations from the surrounding natural environment. Based on current scientific information, 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 2.

### 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 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 was 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 fungus Aphanomyces astaci, which has eliminated native freshwater crayfish from many river systems in Europe (FAO 2007; OIE 2019g). 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 2019k). 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, yellowhead 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 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  $1.21 \times 10^3$  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. The Australian prawn farming industry produces over 4,500 tonnes (2018-19) of product annually with a value estimated at over \$80 million (2018-19) (Gippel 2020; State of Queensland 2020). Currently 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). Significant expansions in prawn aquaculture productions are planned across Australia.

In Queensland, the main prawn aquaculture state, during 2018-19 there were 20 producing prawn farms and production in the prawn sector was 4630 tonnes, valued at \$80.4 million. Hatchery sales of prawns for 2018-19 were worth \$0.9 million and 388 million postlarvae were produced (State of Queensland 2020). The farms are situated singly or in small groups along the State's approximately 2000 km eastern coastline. During 2018-19 in New South Wales, 164 tonnes of *P. monodon* was produced valued at \$3.37 million (Gippel 2020). Farming of *P. monodon* accounts for the majority of prawn farming production in Australia. Aquaculture contributes 18% of total Australian prawn production volume (Mobsby & Curtotti 2020). It is noted that 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 at a national level.

Wild-caught prawns were worth \$280 million in 2017–18 (Mobsby & Curtotti 2020). Other commercially important crustacean species for fishery and aquaculture production include freshwater crayfish, marine lobsters and crabs. Rock lobster, in 2017-18, contributed 39.8% (\$713 million) to the wild-caught gross value of fishery production. Crabs are also one of the major species groups harvested from inshore and coastal Australian waters, and production reached \$30 million in 2017-18 (Mobsby & Curtotti 2020). Freshwater 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 \$1.4, \$3 and \$1.2 million in 2017-18, respectively (Mobsby & Curtotti 2020).

This draft 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.

The Australian Government provided \$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 \$17 million on the operational response to WSD and committed a further \$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 Logan River prawn farming industry production losses in 2016–17 were estimated to be approximately

\$23.5 million (excluding their response costs), the impact of the movement restriction area in the fisheries industry was estimated to be of \$20.5 million and it was estimated that the cost of lost hatchery and breeding stocks to be approximately \$5–6 million (Ridge Partners 2017). Of these costs, the Australian and Queensland governments reimbursed or will reimburse \$21.5 million and pledged a further \$30 million for concessional loans.

Further, the Australian Government provided financial support so that affected farms could remain destocked for 18 months. Prawn farmers will need to repay up to \$4 million through an industry levy. The Australian Government also provided a \$5 million assistance package to the Moreton Bay fishery industry, who continue to be affected by the WSD outbreak in South-East Queensland.

Commercial fishing industries subject to closures or movement restrictions and 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.

Following the detection of WSSV in prawn farms in 2016, movement restrictions of high risk crustaceans and marine worms from the affected area in South-East Queensland were put in place and some jurisdictions implemented interstate movement restrictions of live crustaceans and crustacean products pending eradication of the hazard. In the WSSV eradication program, the challenge was establishing and enforcing the movement restriction area in South-East Queensland in a timely and efficient manner to ensure the virus did not spread further. Due to the wide host range of WSSV and its ability to be transmitted by various routes, if WSSV had continued to spread up and down the east coast of Australia, eradication would be impossible. To date, WSSV has not been detected outside of the South-East Queensland movement restricted area.

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 draft 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 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, etc.). 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).

### 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 twoperson 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 Australia's Logan River in 2017 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 2017–18, Australia exported more than 5300 tonnes of prawns (from both aquaculture and wild fisheries sectors) valued at \$90 million (Mobsby & Curtotti 2020). The major prawn export destinations for Australia in 2017–18 were Japan (918 tonnes valued at \$23.6 million), Hong Kong (948 tonnes valued at \$18.5 million) and Vietnam (1,290 tonnes valued at \$15.4 million) (Mobsby & Curtotti 2020).

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, IHHNV, IMNV, TSV, YHV, *M. rosenbergii* nodavirus, *A. astaci* (Han et al. 2019b) and DIV1 (World Trade Organization 2020b). Requirements for AHPND and NHP will take effect from January 2021 (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 require 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.

Recently, 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 2020c).

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 OIE Code recognises the concept of zoning (regionalisation) and compartmentalisation (OIE 2019f). Zoning would require additional specific regulatory measures such as movement restriction 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 5) (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 draft 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.

Category	Species name	Common name(s)		
Critically endangered	Cherax tenuimanus	hairy marron, Margaret River hairy marron, Margare 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		

Table 5 Crustacean species that are critically endangered, endangered or vulnerable in Australia

*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 restriction 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.

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.

# 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 5. This was done by determining which of the shaded cells with bold font in Figure 5 corresponded to the level and magnitude of the particular impact.

The following were considered during this process:

- 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.

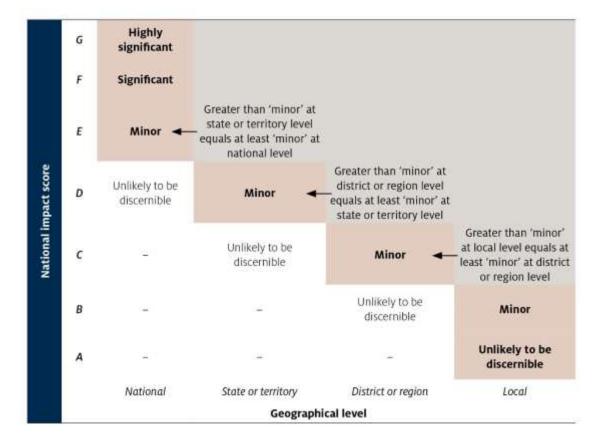


Figure 5 Assessment of direct and indirect impacts on a national scale

#### 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 6. 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.

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

Table 6 Rules for combining direct and indirect impacts

# 4.5.7 Determination of likely consequences for outbreak scenario

'Likely consequences' for the outbreak scenario were determined by using the matrix in Figure 6 to combine the 'overall impact' (see <u>Step 2: Combining direct and indirect impacts</u> in section 4.5.6) with the 'likelihood of establishment and spread' (see section 4.5.4 <u>Estimation of partial</u> <u>likelihood of establishment and spread</u>).

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.

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	

Figure 6 Matrix for determining the 'likely consequences' for the outbreak scenario

# 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 <u>Determination of the partial</u> <u>annual likelihood of entry and exposure</u>) with the estimate of 'likely consequences' (see section 4.5.7 <u>Determination of likely consequences for outbreak scenario</u>) using the risk estimation matrix (Figure 7).

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.

Likelihood of entry	Consequences of entry and exposure ('likely consequences')						
and exposure (PALEE)	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	

#### Figure 7 Matrix for determining the partial annual risk of exposure

### 4.6.2 Estimation of overall annual risk

The overall annual risk is obtained by combining the PAR (see section 4.6.1 <u>Determination of partial annual risk</u>) for each of the exposure groups using the six rules outlined in Table 7.

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.

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

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 <u>Options for biosecurity management of imported prawns</u>) 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.

#### **Options for biosecurity management of imported prawns** 5

Biosecurity measures considered in this draft 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.

<u>Appendix 3</u> 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 draft 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 (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. In some cases, it might be necessary for the disease to be subject to compulsory reporting or be the subject of consideration in disease investigation. The OIE Aquatic animal health code (OIE Code) chapter 4.1 'Zoning and compartmentalisation' (OIE 2019f), chapter 1.4, Article 1.4.6 '*Pathways to demonstrate freedom from disease*' (OIE 2019c) and the relevant provisions in each disease chapter of the OIE 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 visit (conducted in person, or virtually—noting current travel restrictions in place worldwide) of the proposed free country, compartment or zone, before the system could be approved. It is still considered that importation from 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 free countries, compartments or zones is expected to eliminate the entry risk of hazards. 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. Therefore, an in-depth case-by-case assessment needs to be undertaken which considers the hazard(s), the country, compartment or zone and other relevant information.

#### 5.1.2 Sourcing from wild stocks

The Prawn IRA 2009 considered allowing imported prawns that were sourced from wild-caught populations which had been tested and found free of hazards (subject to verification by the overseas competent authority), as this option would reduce the likelihood of entry.

The introduction of species restrictions (where only species not farmed are permitted) were also considered. The Prawn IRA 2009 considered that the effectiveness of such measures would depend on the hazard, as well as the practicality of ensuring compliance with respect to prawn species identification. Ensuring prawns were wild-caught by restricting imports to prawns that have been caught, processed and packed on-ship, again were contingent on the practicality of ensuring compliance was also considered.

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 competent authority attestation to this effect. The department also 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.

Consideration of alternative biosecurity measures for prawns sourced from wild stocks will require a case-by-case assessment (in-line with that discussed in <u>section 5.1.2</u>). A submission, which includes supporting scientific information that explains the extent to which the alternative measures would achieve Australia's ALOP, should be provided to the department for consideration.

#### 5.1.3 Cooking

The Prawn IRA 2009 considered that whole prawns could be permitted import subject to cooking off-shore in a premises approved by, and under the control of, the competent authority. Alternatively, prawns could be cooked post-arrival, under biosecurity control in Australia. Prawns are usually cooked whole, and the cephalothorax and shell are removed before consumption of tail meat (abdominal muscle) (ADVS 1999).

Cooking would be expected to cause some inactivation or reduction in the titre of most hazards including viruses and bacteria (OIE 2019m) and significantly reduce the likelihood of imported prawns being used as bait, berley or crustacean feed or being further processed in Australia (outside of an approved arrangement). A prawn is considered fully cooked when all the protein is coagulated. It is at this point that marketability remains, there has been some pathogen inactivation and the attractiveness of the prawn for other end-uses is significantly reduced. For example, cooking a prawn to a minimum 70°C core temperature for at least 11 seconds is sufficient to achieve coagulation of all protein.

For all the protein to be coagulated in a whole prawn under commercial conditions depends on the size and quality of the prawn. Winkel (1998), in an evaluation of the cooking process for Australian farmed *P. monodon*, recommended that prawns be cooked to a core temperature of 85°C so that the product is marketable (that is, completely cooked, not chewy, no black spot and aesthetically acceptable). 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.

Cooking prawns in boiling water for periods such as those used by seafood processors and recommended by relevant guidelines and advisory notes may be sufficient to reduce the infectious titre of some prawn pathogens (for example, infectious myonecrosis virus). However, standard commercial cooking practices may not completely inactivate some viruses of concern such as Taura syndrome virus.

The Prawn IRA 2009 considered that when combining the reduced likelihood of inappropriate end-use of cooked prawns with the expected pathogen-specific inactivation by commercial cooking, that cooking would reduce the overall risk to an acceptable level. It is considered that cooking has a significant impact on the likelihood of exposure because uncooked prawns are the preferred option for use as bait, berley or crustacean feed. This assumption may be revisited subject to the outcomes of the bait and berley survey.

In summary, cooking would generally be expected to reduce both entry and exposure risks 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 necrotising hepatopancreatitis bacteria. 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. This assumption may be revisited subject to the outcomes of the bait and berley survey. 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 draft 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.

## 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 decreased 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, provided they have been par-cooked. Par-cooking reduces the likelihood of diversion of the product to bait and berley as well as 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 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 <u>Proposed</u> <u>biosecurity measures for imported prawns</u>) 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.

The biosecurity risks associated with dumpling and dim sum-type products which contain uncooked prawns are managed due to the decreased 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 <u>Proposed biosecurity measures for imported prawns</u>) 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 completely 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. However, because current data are not available about whether head and shell removal still significantly reduces the likelihood of product being used as bait or berley, the extent to which this option would reduce entry and exposure risks will depend on the hazard of concern and the exposure pathway. Current data does show that convenience is the main driver for recreational fishers

purchasing supermarket prawns for use as bait or berley (Biosecurity Queensland 2017; Kantar Public 2017, 2019). The 2007 survey identified an increase in the use of peeled prawns to bait fishing hooks (Kewagama Research 2007). This draft 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.

Once available, the department will use data from the National social and economic recreational fishing survey to amend the risk assessments and the draft report, including the effectiveness of head and shell removal as a biosecurity measure, if appropriate.

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 risks 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 gutassociated 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 <u>Appendix 4</u> 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 100% secure seals intact 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). Visual inspection of prawns during on-arrival sampling for WSSV and YHV1 testing is considered unlikely to reduce the risks associated with other hazards entering Australia. Prawns at this point in the supply chain are less likely to show visible signs of disease, compared to whole prawns post-harvest, because they are frozen (and will have had the head and shell removed). Additionally, prawns that are free of external clinical signs (for example, subclinical infection) or that have subtle lesions are likely to pass post-harvest inspection.

Testing methods should be at least to a standard consistent with the recommendations in the latest version of the OIE *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 OIE, 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 draft risk review, pre-export testing in the country of origin is not considered equivalent to on-arrival testing in Australia. This is because for the purposes of considering this biosecurity measure it is assumed that Australia has not assessed the exporting country's pre-export testing systems.

Options for equivalence would be considered on a case-by-case basis. 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. Systems outside those considered within this draft risk review will be assessed on a case-by-case basis.

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. The department considers that the application of an on-arrival compliance based inspection program in Australia will be required for any batch testing 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.

#### 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, that 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. Any reduction in unintended end-use or deliberate diversion, for example as bait, is beneficial in reducing risk. Australian state and territory governments could also consider implementing 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 <u>biosecurity labelling requirements for uncooked prawns</u>.

#### 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.

#### Sourcing from non-emergency harvested stock 5.2.2

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.

#### Minimum size 5.2.3

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.

### 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 2019h).

"*Ca.* H. penaei" is an obligate intracellular bacterium of the order *Rickettsiales* (Nunan et al. 2013). Susceptible host species include various penaeid prawns (OIE 2019h). NHP was first reported in farmed prawns from Texas, United States of America (USA) in 1985 and has since spread 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 (OIE) (OIE 2020b) and is on Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). "*Ca.* H. penaei" is 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). This chapter will refer to NHPB where that name was used in the cited literature, otherwise, "Ca. H. penaei" will be used.

### 6.2 Technical information

The following technical information 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). More recently 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 ( $0.25 \times 0.9 \mu$ m) and a motile helical variant with eight flagella located at the basal apex ( $0.25 \times 2-3.5 \mu$ m). 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).

NHPB 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, NHPB 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). NHPB 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 NHPB 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 NHPB to retain infectivity (Gracia-Valenzuela et al. 2011). Additional studies were able to reproduce NHP by using prepared homogenates composed of NHPB-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 NHPB was transmitted to juvenile *P. vannamei* fed on NHP-affected hepatopancreas, but only when flash frozen at -80°C, and that infectivity of NHPB 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 NHPB-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 NHPB, obtained by density gradient ultracentrifugation and preserved at –70°C (Frelier, Loy & Kruppenbach 1993).

NHPB 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 NHPB 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 NHPB 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 OIE *Aquatic animal health code* (OIE Code) (OIE 2019b) include:

Penaeus vannamei <sup>N, E</sup> (Brinez, Aranguren & Salazar 2003; Crabtree et al. 2006; Frelier et al. 1992; Krol, Hawkins & Overstreet 1991; Lightner & Redman 1994; OIE 2019h; 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 2019h)
- Penaeus duorarum N (Aguirre Guzman et al. 2010; OIE 2019h)
- Penaeus marginatus <sup>№</sup> (Brock et al. 1986a; OIE 2019h)
- Penaeus merguiensis N (Brock et al. 1986a; Lightner & Redman 1985; OIE 2019h)
- *Penaeus monodon* <sup>E</sup> (OIE 2019h; Pantoja & Lightner 2003)
- Penaeus setiferus N, E (Frelier et al. 1994; OIE 2019h)
- *Penaeus stylirostris* <sup>№</sup> (Lightner & Redman 1994; OIE 2019h).

NHPB-positive PCR results (E = experimental exposure) have been reported in the following species:

• Homarus americanus E (Ávila-Villa et al. 2012b; OIE 2019h).

NHPB-positive PCR results and necrotic spots in the hepatopancreas of the lobster *H. americanus* were found after forced feeding with NHPB (Ávila-Villa et al. 2012b). Based on these results, (Ávila-Villa et al. 2012b) suggested that NHPB 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 NHPB (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 2019h).

#### **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 NHPB 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) 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 NHPB 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, NHPB 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 NHPB 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 NHPB 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). NHPB 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 NHPB-positive females were also NHPB-positive, suggesting transmission from broodstock to progeny occurs. Transmission has also been demonstrated through injection of purified bacteria (Frelier, Loy & Kruppenbach 1993).

No NHPB vectors are currently known in natural infections (OIE 2019h). However, *Navicula* sp., *Artemia* sp. and zooplankton have been proposed. NHPB has been detected in samples of zooplankton from areas with high NHP prevalence by qPCR but it is still unknown whether the NHPB is able to colonize the zooplankton or it is associated with chitin-containing surfaces (Mendoza-Cano et al. 2013). NHPB has been detected by PCR in *Navicula* and *Artemia franciscana* experimentally exposed to NHPB. Of those prawns fed on NHPB-positive *Navicula*, 20% were found to be NHPB-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 NHPB (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. NHPB, 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 NHPB 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 NHPBinfected hepatopancreas. The amount of NHPB 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 NHPB-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 NHPB-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 NHPB-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 NHPB 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. NHPB-infected female broodstock is also reported to produce nauplii and larva of decreased quality (Aranguren et al. 2006).

#### **Tissue tropism**

NHPB targets the hepatopancreas with infection reported in all hepatopancreatic cell types (Lightner et al. 2012b). NHPB 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 NHPB (Lightner & Redman 1994). NHP was quantified by qPCR in *P. vannamei* (mean weight 5.1g) fed 1 piece of NHPB-infected hepatopancreas (0.05g piece, with an undetermined copy number of NHP). NHPB was detected at 10<sup>3</sup>–10<sup>7</sup> copies/mg in hepatopancreas and 10<sup>1</sup>–10<sup>5</sup> copies/mg in faeces (Vincent & Lotz 2005). Lethal infections contained 10<sup>6</sup>–10<sup>7</sup> copies/mg in hepatopancreas and 10<sup>3</sup>–10<sup>6</sup> copies/mg in faeces. The amount of NHPB present in the hepatopancreas was higher than that observed in faeces of the same individual (Vincent & Lotz 2005). In a separate study, quantification of NHPB in hepatopancreas and faeces samples of *P. vannamei* (mean weight 2.8g) by qPCR showed that NHPB copy number

ranged from  $3.0 \times 10^2 - 8.8 \times 10^7$  copies/µg of DNA in hepatopancreas, and mean copy number of  $4.3 \times 10^3 - 4.2 \times 10^6$  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 2019h).

#### 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 2019h). 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 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 2019h). 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 2019h).

#### Testing

Chapter 2.2.3 of the OIE *Manual of diagnostic tests for aquatic animals* (OIE Manual) (OIE 2019m) 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 OIE recommended method for targeted surveillance to declare freedom from "*Ca.* H. penaei" (OIE 2019h). Recently, 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 OIE 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 2019h). NHP, particularly in the initial phase, can be treated by using antibiotics in medicated feeds (OIE 2019h). NHPB 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 2019h).

#### 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 in 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 NHPB 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 NHPB 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 <u>General considerations and risk assessment process</u> and the technical information about "*Ca.* H. penaei" presented in this chapter, the following 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 <u>Appendix 3</u>.

#### 6.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for *"Ca.* H. penaei".

- This draft 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 2, the annual likelihood of entry of "*Ca.* H. penaei" in imported prawns was estimated to be **very low**.

#### 6.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for *"Ca.* H. penaei".

- *"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 were considered unlikely to be directly exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent their introduction either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude "*Ca.* H. penaei" or imported prawn wastes.

- 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, although the host range is relatively narrow and 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. 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 2, 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 4 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 following were considered relevant when determining the partial likelihood of establishment and spread for "*Ca.* H. penaei".

- *"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 greater for farmed and hatchery crustaceans than for wild crustaceans. This is due to the stressors 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 less 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 2, the partial likelihood of establishment and spread of "*Ca.* H. penaei" in each exposure group for the outbreak scenario (refer section 4.5.1 <u>Identification of the outbreak scenario</u>) was estimated to be:

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

#### Determining adverse impacts resulting from the outbreak scenario

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

#### 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* sp. 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 OIE 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 restriction area were put in place for an outbreak of "*Ca.* H. penaei", there would be on-going 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 an OIE-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 restriction 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 8 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of "*Ca.* H. penaei". The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

Table 8 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	А

Effects	Criteria	Level	Impact	Score
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	Е
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	District or region	Minor	С
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	А
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	В

#### 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 6) 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 7 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 7.

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 <u>Appendix 3</u>).

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 spread throughout Asia and to Ecuador and Mexico (Flegel 2015; 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 (OIE) (OIE 2020b) nor is it included in Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). Infection with CMNV is included in the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2019a). CMNV is exotic to Australia.

### 7.2 Technical information

The following technical information 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 RNAdependent RNA polymerase gene of CMNV classifies the nodavirus as a member of the genus *Alphanodavirus*, in the family *Nodaviridae* (Xu et al. 2020a; 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  $-70^{\circ}$ C (Tang et al. 2007b). MrNV can survive freezing at  $-20^{\circ}$ 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:

- *Corophium sinense Zhang* <sup>N</sup> (amphipod) (Liu et al. 2018b)
- Diogenes edwardsii N (hermit crab) (Liu et al. 2018b)
- *Exopalaemon carinicauda* N, E (Liu et al. 2017)
- Machrobrachium rosenbergii N (Zhang et al. 2017b)
- *Mugilogobius abei* <sup>N</sup> (finfish) (Zhang et al. 2018)
- Ocypode cordimundus <sup>N</sup> (ghost crab) (Liu et al. 2018b)

- Paralichthys olivaceus <sup>N</sup> (finfish) (Wang et al. 2018)
- *Parathemisto gaudichaudi* <sup>N</sup> (amphipod) (Liu et al. 2018b)
- Penaeus chinensis <sup>N</sup> (Zhang et al. 2014; Zhang et al. 2017b)
- *Penaeus japonicus* <sup>№</sup> (Zhang et al. 2014; Zhang et al. 2017b)
- Penaeus monodon <sup>N</sup> (Zhang et al. 2017b)
- *Penaeus vannamei*<sup>N, E</sup> (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* sp. <sup>№</sup> (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* <sup>№</sup> (common clam) (Liu et al. 2018b)
- unidentified gammarid amphipod <sup>№</sup> (Liu et al. 2018b).

*C. gigas, A. sinica and Balanus sp.* 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 2015). 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).

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 2015).

#### 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).

#### 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 sp.* (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<sup>3</sup>) 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

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).

#### Tissue titre

One study that examined the titre of CMNV in infected *P. vannamei* found that the viral loads varied from  $1.5 \times 10^2$ – $6.7 \times 10^6$  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– $6.5 \times 10^6$  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– $3.5 \times 10^4$  copies/mg tissue (examined by real-time RT-LAMP), which was lower than the viral load in the muscles of *P. vannamei* ( $2.1 \times 10^1$ – $8.3 \times 10^5$  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 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* 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; Zhang et al. 2018).

#### 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 surrounding coastal waters near the drainage channel of a prawn farm (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 limited reports of CMNV infecting finfish species. Because none of these finfish species is reported in Australia, finfish species have not been considered in this risk assessment. However, related species are native to Australia (for example, Mugilogobius (Fishes of Australia 2015a), family Paralichthyidae (Fishes of Australia 2015b) and family Gobiidae (Bray 2017)). 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 <u>General considerations and risk assessment process</u> and the technical information about CMNV presented in this chapter, the following risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of CMNV meets Australia's ALOP are shown in <u>Appendix 3</u>.

#### 7.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for CMNV.

- This draft 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 39% prevalence in the wild marine fish collected from 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.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 2, the annual likelihood of entry of CMNV in imported prawns was estimated to be **high**.

#### 7.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for CMNV.

- 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.
- It is noted that CMNV has been reported to also affect three finfish species. Should information become available that suggests non-crustacean species native to Australia are susceptible to CMNV, the department will reconsider the risk assessment for CMNV.

- 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 were considered unlikely to be exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent the introduction of imported prawns either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude CMNV or imported prawn wastes.
- 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 CMNV 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 CMNV.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 2, the partial likelihood of exposure of each exposure group to CMNV in imported prawns was estimated to be:

- Farmed crustaceans—Low.
- Hatchery crustaceans—Low.
- Wild crustaceans—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 4 and was found to be:

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

#### 7.3.4 Consequence assessment

#### Partial likelihood of establishment and spread

The following were considered relevant when determining the partial likelihood of establishment and spread for CMNV.

- CMNV can be transmitted by ingestion of infected tissues, co-habitation, 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, 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*.
- The likelihood of CMNV establishment, following a given quantity of CMNV entering the environment of an exposure group, is greater for farmed and hatchery crustaceans than for wild crustaceans. This is due to the stressors 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 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 less 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.
- 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 2, the partial likelihood of establishment and spread of CMNV in each exposure group for the outbreak scenario (refer section 4.5.1 <u>Identification of the outbreak scenario</u>) was estimated to be:

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

#### Determining adverse impacts resulting from the outbreak scenario

The following were considered relevant when determining the adverse impacts resulting from establishment and spread of CMNV.

#### 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 no reports of CMNV in wild prawns and no reports of declines in catch rates or associated mortalities.
- Significant impacts have been reported in China where CMNV is present. 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 and other marine species are known to be susceptible to CMNV. If CMNV infection spreads to native 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 OIE 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 2018). 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 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 restriction area were put in place for an outbreak of CMNV, there would be on-going 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 restriction areas put in place would have indirect impacts on other industries such as seafood suppliers and commercial wild catch fisheries due to the broad host range of CMNV.
- 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.
- 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.
- 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.
- 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 restriction 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 9 shows the individual impact scores for each criteria (determined using Figure 5) for the establishment and spread of CMNV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

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

#### Table 9 Overall impact of establishment and spread of CMNV for the outbreak scenario

#### 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 6) and found to be:

- Farmed crustaceans—Moderate.
- Hatchery crustaceans—Low.
- Wild crustaceans—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 7 and found to be:

- Farmed crustaceans—Low.
- Hatchery crustaceans—Very low.
- Wild crustaceans—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 7.

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 <u>Appendix 3</u>.

The factors considered and the conclusions reached are presented below.

#### 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 following were considered:

- 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.
- 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 head and shell off prawns as feed in research or public aquaria.
- Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal. 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 prawns with the biosecurity measure, head and shell removal applied was determined to be **very low.** 

#### 7.4.2 Cooking

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

- 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).
- It is assumed cooking may reduce, but not completely inactivate CMNV in imported prawn tissues and sufficient viable virus to cause disease may still be present. Therefore, cooking is not expected to reduce the likelihood of entry.
- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will also reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017, 2019). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed prawns being directly exposed to cooked prawns through the inlet channels is negligible.

#### Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, 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 following were considered:

- 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 <u>Value-added products</u>). The likelihood of entry of CMNV 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 CMNV in the product more than head and shell removal does.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.
- The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

Based on this information, the overall restricted risk of imported 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 recently 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 2020a). DIV1 has been detected by PCR in grossly normal wild prawns caught from the Indian Ocean (Srisala et al. 2020a).

DIV1 is included in the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2020b). Infection with DIV1 is proposed for inclusion as a disease notifiable to the World Organisation for Animal Health (OIE) and is proposed to be included in Australia's *National list of reportable diseases of aquatic animals*. DIV1 is exotic to Australia.

To simplify naming of the hazard in this chapter, SHIV and CQIV will be referred to as DIV1, even if the literature being cited referred to the individual isolate names.

#### 8.2 Technical information

The following technical information 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 percentages of 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). It has also been reported that an NCBI BLAST analysis identified the 34 amino acid sequence excluding the primer regions to be most identical (55%) to 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 et al. 2017; Qiu et al. 2018a).

No other reports on the stability of DIV1 were found. However, 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). 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 2019n).

#### 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* <sup>E</sup> (Chen et al. 2019a)
- *Macrobrachium nipponense* <sup>№</sup> (Qiu et al. 2019a)
- Macrobrachium rosenbergii N (Qiu et al. 2019a)
- Penaeus vannamei <sup>N, E</sup> (Qiu et al. 2017; Qiu et al. 2018a)
- Penaeus monodon N (OIE 2020a; Srisala et al. 2020a).
- *Procambarus clarkii* <sup>N, E</sup> (Qiu et al. 2019a; Xu et al. 2016).

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

- Antarctic krill <sup>E</sup> (China Fisheries Channel 2020; NACA 2020b)
- *Eriocheir sinensis* <sup>E</sup> (crab) (Pan et al. 2017)
- 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 japonicus* N (Qiu et al. 2019a; Qiu et al. 2018c)
- *Penaeus merguiensis* <sup>E</sup> (Liao et al. 2020)

DIV1-positive PCR results have been reported in the following species, but no active infection was found (N= natural exposure)

• Cladocera N species (water flea) (Chen et al. 2019a; Qiu et al. 2019a).

*Cladocera* spp. is not considered a DIV1 susceptible species (Chen et al. 2019a), although it may act as a vector.

Recently, it was 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 hours (Liao et al. 2020). It was not investigated if there were any changes consistent with DIV1 infection or associated mortality, 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 DIV infection in *P. merguiensis* (Liao et al. 2020).

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). Mortalities associated with infection with DIV1 in farmed *P. monodon* have been reported in Taiwan (OIE 2020a). 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). Recently, infection with DIV1 in farmed *C. quadricarinatus, P. vannamei* and *P. monodon* was reported in Taiwan (Cheng 2020; Chung 2020; OIE 2020a; Su-min, Shen & Yi-ching 2020).

#### Prevalence

Surveys from farmed stocks in provinces of China have reported a prevalence of infections 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 Taizhou, Zhejiang provinces in China found 25.7% (83/323) were positive for DIV1 by qPCR (Qiu et al. 2018a).

There is only one report of DIV1 in the wild where it was 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).

#### 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). It has been reported that 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 a recent outbreak of DIV1 in China was due to over wintered, pond-reared broodstock which had contracted DIV1 and passed it through to the first crop (Harkell 2020b). Taiwan reported mortality rates of 20% in a *P. monodon* farm, and 0%, 20% and 90% in three DIV1 infected *P. vannamei* farms (OIE 2020a).

#### 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). It is therefore expected that horizontal transmission through ingestion of infected tissue occurs. 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). A recent outbreak in Taiwan was speculated to be due to ponds being infected by migratory birds, 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 2019n).

#### 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 not known. *P. vannamei* (6cm) fed once with minced DIV1-infected tissues (5mm<sup>3</sup>) at 5% of total body weight became infected with DIV1 (Qiu et al. 2017). Cumulative mortalities reached 100% within 2 weeks of post-oral administration. 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  $50 \pm 26.5\%$  following feeding with 3g DIV1-infected cephalothoraxes of *P. vannamei* with a viral load of about 10<sup>10</sup> copies/g (Chen et al. 2019a).

Intramuscular injection with  $2\mu$ /g body weight of a 1:10<sup>6</sup> dilution of purified DIV1 resulted in lethal infections in *P. vannamei, C. quadricarinatus* and *Pr. clarkii* (Xu et al. 2016). Intramuscular injection of 50µl of a supernatant containing  $1.5 \times 10^5$  copies/µg DIV1 DNA into *P. merguiensis* resulted in a number of differentially expressed genes, included 13 immune-related genes, 48 hours post-exposure (Liao et al. 2020).

#### 8.2.3 Pathogenesis

#### 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  $25.4 \pm 16.9\%$ ). Hepatopancreas and muscle contained the lowest DIV1 loads (relative abundance of  $2.44 \pm 1.24\%$  and  $2.44 \pm 2.16\%$ , respectively) (Qiu et al. 2019a).

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

#### 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). 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 typical white triangle, infection with DV1 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.

#### 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).

### Testing

A qPCR assay that targets the major capsid protein (MCP) has been developed to detect and quantify DIV1 (Qiu et al. 2020). Also, a real time isothermal recombinase polymerase amplification assay that targets the MCP gene of DIV1, was developed for field diagnosis (Chen et al. 2019b). An *in situ* hybridization protocol that targets a region of the MCP gene of DIV1 is also publicly available (Qiu et al. 2017).

# 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 DIV infection in *P. merguiensis* (Liao et al. 2020) and one report of 20% mortality in farmed *P. monodon* (OIE 2020a). In Taiwan, DIV1 was detected in farmed *C. quadricarinatus, P. vannamei* and *P. monodon* (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 proposed to be included in Australia's *National list of reportable diseases of aquatic animals*. Based on the preceding information, risk assessment 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, although the severity and clinical signs of disease is unknown. The mortality rate in farmed *P. monodon* with DIV1 infection suggested 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 or 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 <u>General considerations and risk assessment process</u> and the technical information about DIV1 presented in this chapter, the following 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 <u>Appendix 3</u>.

# 8.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for DIV1.

- This draft 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.
- 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 2, the annual likelihood of entry of DIV1 in imported prawns was estimated to be **high**.

#### 8.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for DIV1.

- 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. 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 were considered unlikely to be directly exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent their introduction either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude DIV1 or imported prawn wastes.
- 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 2, the partial likelihood of exposure of each exposure group to DIV1 in imported prawns was estimated to be:

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

# 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 4 and was found to be:

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

### 8.3.4 Consequence assessment

### Partial likelihood of establishment and spread

The following were considered relevant when determining the partial likelihood of establishment and spread for DIV1.

- 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 may be spread by vectors. Species present in Australia that may act as vectors include *Cladocera* species and polychaetes.
- 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*.
- It is unknown if *P. merguiensis* are susceptible to clinical signs or disease from infection with DIV1.
- There is one report of 20% mortalities attributable to DIV1 in *P. monodon*. The report did not identify whether the prawns had clinical signs of disease.
- The likelihood of DIV1 establishment, following a given quantity of DIV1 entering the environment of an exposure group, is greater for farmed and hatchery crustaceans than for wild crustaceans. This is due to the stressors 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 considered to be 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 less which reduces the opportunities for transmission. 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. Establishment of DIV1 in wild populations may result in spread to its natural geographic limits. Although based on the available information about DIV1, this is considered less likely than for hazards with larger host ranges such as yellow head virus genotype 1 (YHV1) which reduces the likelihood of spread.
- 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 and polychaetes. *C. quadricarinatus* are susceptible to DIV1 and may be capable of entering farms through movement across

short distances of land. However, this is considered less likely than for species such as crabs, given the physiological and physical requirements of *C. quadricarinatus*. There are no crab species present in Australia which are known to be susceptible to DIV1.

- 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.
- Once established and based on the available information, DIV1 spread from farms to wild populations is less likely than for other hazards, for example, YHV1, with broader host ranges. However, some 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 may be susceptible to infection. It is unclear if *P. monodon* 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 2, the partial likelihood of establishment and spread of DIV1 in each exposure group for the outbreak scenario (refer section 4.5.1 <u>Identification of the outbreak scenario</u>) was estimated to be:

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

#### Determining adverse impacts resulting from the outbreak scenario

The following were considered relevant when determining the adverse impacts resulting from establishment and spread of DIV1.

#### Direct effects

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

- *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 recent report of 20% mortality in farmed *P. monodon* was found. 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 DIV 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 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* and *P. monodon.*
- 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.
- 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 included in the *List of diseases in the Asia-Pacific*. Infection with DIV1 is proposed for inclusion as a disease notifiable to the OIE and in *Australia's National list of reportable diseases of aquatic animals*. If included, 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 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 restriction 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 and freshwater and marine crustacean industries may be indirectly affected by movement restriction areas that encompass potential DIV1 susceptible species.
- 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

- DIV1 is proposed for listing by the OIE. 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, 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*.
- *Cherax tenuimanus* is listed as critically endangered. If DIV1 were able to cause disease in *C. tenuimanus* it could result in a significant impact on the survival of that already endangered species. It is unknown if *C. tenuimanus* would be affected by DIV1.
- In light of 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 restriction 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 10 shows the individual impact scores for each criteria (determined using Figure 5) for the establishment and spread of DIV1. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Minor	Е
	The environment (native animals/plants, and non-living environment)	Local	Minor	В
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	Е
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D

Table 10 Overall impact of establishment and spread of DIV1 for the outbreak scenario

Effects	Criteria	Level	Impact	Score
	Economic (international trade effects)	District or region	Minor	С
	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	В

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 6) and found to be:

- Farmed crustaceans—Moderate.
- Hatchery crustaceans—Low.
- Wild crustaceans—Very 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 7 and found to be:

- Farmed crustaceans—Low.
- Hatchery crustaceans—Very low.
- Wild crustaceans—Very 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 7.

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 <u>Appendix 3</u>.

The factors considered and the conclusions reached are presented below.

#### 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 following were considered:

- 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.
- 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 head and shell off prawns as feed in research or public aquaria.
- Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal. Additionally, the very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels remains.

Based on this information, the overall restricted risk of imported 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 following were considered:

- No reported investigations into the stability of DIV1 to heat treatments were found. However, another member of *Iridoviridae*, RSIV, has been reported to be inactivated at 56°C for 30 mins (OIE 2019n). 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) If DIV1 is comparable to RSIV, then a sufficient infectious dose of DIV1 would likely remain in the prawns after cooking as this may be above the 60°C for 1 min or 70°C for 11 seconds used to cook prawns.
- Given the uncertainty regarding the effect of cooking on DIV1 viability, it is assumed that cooking may reduce the load of infectious DIV1 in imported prawn tissues but not completely inactivate it. It is assumed that some infectious virus will remain. Therefore, cooking is not expected to reduce the likelihood of entry.
- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will also reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017, 2019). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed prawns being directly exposed to cooked prawns through the inlet channels is negligible.

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, applied was determined to be **negligible**.

#### 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 following were considered:

- 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 <u>Value-added products</u>). The likelihood of entry of DIV1 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 DIV1 in the product more than head and shell removal does.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.
- The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

Based on this information, the overall restricted risk of imported 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).

EHP is not listed as notifiable to the World Organisation for Animal Health (OIE) (OIE 2020b). EHP is on Australia's *National list of reportable diseases of aquatic animals* (AHC 2018) and the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2020b). EHP is exotic to Australia.

# 9.2 Technical information

The following technical information 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 ovoid spores (Tourtip et al. 2009). EHP spores are approximately 1  $\mu$ m 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<sub>4</sub> 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).

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 in tissue homogenate was reported to survive freezing at -20°C (Karthikeyan & Sudhakaran 2019). In contrast, storing minced EHP-positive hepatopancreas and inoculum at -80°C with glycerol was reported to inactivate 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.

Differences in temperature tolerance between species of microsporidia have been reported. For example, spores of Encephalitozoon cuniculi heated at 100°C for 1 min failed to grow in cell culture, whereas spores of Encephalitozoon intestinalis and Encephalitozoon hellem had to be heated for 5 and 10 mins at 100°C, respectively, for 100% inhibition of growth (Li & Fayer 2006). E. intestinalis and E. hellem spores in water without cryoprotectants were held at -20°C for 24 hours, and *E. cuniculi* spores were held at the same temperature for only 2 hours, to obtain 100% inhibition of growth in cell culture (Li & Fayer 2006). The uncertainty about sensitivity of microsporidian spores to temperature is further complicated as the results obtained in various studies with other species or isolates of microsporidia vary widely. For example, spores of *E. cuniculi* held in water at 4°C for 2 years or at -12°C and -24°C for 1, 8, and 24 hours were infective for mice; those held at  $-70^{\circ}$ C for 1 and 8 hours were much less infective (Koudela, Kucerova & Hudcovic 1999). In contrast, another study reported that spores of E. cuniculi in growth medium held 1 day at -20°C and those held at 4°C for 98 days were not infective (Waller 1979). Still others reported that *E. cuniculi* spores, in growth medium with DMSO and glycerol, held at -70°C or in liquid nitrogen for 6 months infected rabbit choroid plexus cell cultures (Shadduck & Polley 1978).

# 9.2.2 Epidemiology

#### Host range

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

- Penaeus merguiensis (Otta et al. 2016)
- Penaeus monodon N (Tourtip et al. 2009)
- Penaeus stylirostris N (Tang et al. 2015)
- *Penaeus vannamei*<sup>N</sup> (Tangprasittipap et al. 2013).

EHP, or a similar microsporidian within the so-called '*Enterocytozoon* group Microsporidia' (EGM) (Stentiford, Bass & Williams 2019) was suspected to also infect *Penaeus japonicus* (Hudson, Hudson & Pyecroft 2001), but there has been no evidence to confirm the species. Australia has a long history of passive surveillance and a strong system in place and EHP is not considered present.

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, polychaetes and squid <sup>№</sup> (Han, Tang & Kim 2018; Kummari et al. 2018; Tang et al. 2015; Tran 2018).

EHP affects multiple prawn life stages including broodstock and postlarvae (Karthikeyan & Sudhakaran 2019; 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 (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) 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). 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 2019). 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 66% (155/235) and 85% (188/219) of prawn ponds tested across multiple districts in India (Behera et al. 2019; Biju et al. 2016). 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). 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 2019).

#### 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 (Salachan et al. 2017; Tang et al. 2015; Tang et al. 2016).

Transmission of EHP from broodstock to progeny may occur (Vu-Khac et al. 2018). Female *P. vannamei* broodstock experimentally infected (via *per os* challenge and cohabitation) produced nauplius and zoea stages that were EHP-positive, suggesting that EHP can transmit to offspring (Vu-Khac et al. 2018). It is important to consider that EHP transmission may have occurred via contamination of the early larval stages with faeces (containing EHP-spores) from the mother, rather than via true vertical transmission.

*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; 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<sup>3</sup>–4.8 × 10<sup>5</sup> copies/mg (Piamsomboon et al. 2019).

#### 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). The importation of infected frozen prawns may also be a possible route of introduction. EHP DNA has been detected in frozen *P. vannamei* imported into the Republic of Korea from Vietnam and Indonesia (17/58 samples, 29%) (Han et al. 2019b). However, experiments were not conducted to determine if the EHP in the frozen prawns was viable and infectious.

A pathogen described as EHP has also been reported from Venezuela. However, nucleotide sequence comparison of  $\beta$ -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 recently introduced (or at least not recently prior to the detection) to Venezuela from South-East Asia (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.

#### 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). 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. Similarly, 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). More recently, 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  $1 \times 10^7$  copies/ul) and ponds with a history of EHP (about 4×10<sup>4</sup> copies) when compared to ponds where WFS was not present nor any clinical sign of diseases were observed (about  $4 \times 10^2$  copies/ul). This study also reports higher EHP loads in hepatopancreas and faecal strings of prawns from ponds with WFS (average 4×10<sup>7</sup> copies/ul in hepatopancreas, copy number in faecal string not specified) when compared to the ones from ponds without WFS ( $1 \times 10^5$  copies/ul 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). 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) (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) 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 (Sanguanrut et al. 2018; 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 (Otta et al. 2016). Histology of EHP-infected hepatopancreatic tissue revealed various developmental plasmodia stages and mature spores. As infection progresses, EHP has been detected in the gut, heart, abdominal muscle, tail muscle, intestine, leg, nerve, gill and haemolymph of both naturally and experimentally EHP-infected prawns by PCR assays (Karthikeyan & Sudhakaran 2019; Santhoshkumar et al. 2017). Histopathological analysis has shown EHP parasites and spores in the hepatopancreas, muscle, intestines and midgut epithelial cells of infected prawns (Karthikeyan & Sudhakaran 2019; Salachan et al. 2017; Santhoshkumar et al. 2017; Tang et al. 2016).

#### **Tissue titre**

The EHP DNA load in the hepatopancreas of naturally infected frozen *P. vannamei* was quantified by qPCR and ranged from  $1.7 \times 10^2$ – $1.8 \times 10^7$  copies (Han et al. 2019b). The EHP copy number in the hepatopancreas from naturally infected *P. vannamei* (quantified by qPCR) ranged between  $2.5 \times 10^2$ – $1.4 \times 10^8$  copies/mg (Piamsomboon et al. 2019). In faeces samples,  $2.5 \times 10^3$ – $6.9 \times 10^7$  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 only 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 hemocytic 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; 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 (Cai et al. 2018; Kanitchinda et al. 2020; Karthikeyan et al. 2017; Ma et al. 2019; 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 OIE 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).

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).

No reports were found about the impact of EHP on wild prawn populations.

# 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 <u>General considerations and risk assessment process</u> and the technical information about EHP presented in this chapter, the following 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 <u>Appendix 3</u>.

#### 9.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for EHP.

- This draft 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 13–85% in farmed prawns (Kummari et al. 2018; Thamizhvanan et al. 2019). There are no reports of EHP prevalence in wild prawns.
- EHP would be present primarily in the prawn head and gut, although small amounts may be present in the muscle of prawns in the later stages of a very advanced infection.
- 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.
- There is evidence to suggest that freezing prawns at -80°C would reduce the viability of EHP in imported prawns (Mai et al. 2020). However, it is unknown what effect storage at commercial freezing temperatures (-18 to -20°C) would have on EHP viability and

infectiousness. Given the uncertainty regarding the effect of freezing on EHP viability, it is assumed that EHP in imported prawns would survive commercial freezing, storage and transport and remain infectious at the time of import. Should information become available that suggests EHP in prawn tissue is not infectious following commercial freezing, storage and transport, the department will reconsider the risk assessment for EHP.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 2, the annual likelihood of entry of EHP in imported prawns was estimated to be **high**.

#### 9.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for EHP.

- 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 were considered unlikely to be directly exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent their introduction either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude EHP or imported prawn wastes.
- Crustaceans present in hatcheries were considered unlikely to be exposed to imported prawns 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 Australia.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 2, the partial likelihood of exposure of each exposure group to EHP in imported prawns was estimated to be:

• Farmed crustaceans—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 4 and was found to be:

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

#### 9.3.4 Consequence assessment

#### Partial likelihood of establishment and spread (PLES)

The following were considered relevant when determining the partial likelihood of establishment and spread for EHP.

- 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 vectors are present in Australia and include crabs, polychaetes, *Artemia* 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 the stressors 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 EHP 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. Additionally, the densities of susceptible animals are less which reduces the opportunities for transmission. EHP spores can remain infectious in the environment for long periods of time without a host and vectors such as crabs, polychaetes, *Artemia* 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 measures, 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.

- 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 2, the partial likelihood of establishment and spread of EHP in each exposure group for the outbreak scenario (refer <u>Identification of the outbreak scenario</u>) was estimated to be:

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

#### Determining adverse impacts resulting from the outbreak scenario

The following were considered relevant when determining the adverse impacts resulting from establishment and spread of EHP.

#### 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 would not be expected to impact wild fisheries in Australia. There are limited 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 serious effects of EHP 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* species 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

- EHP is not listed as a notifiable disease by the OIE, 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 restriction 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 restriction 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 restriction 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 11 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of EHP. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer <u>Determining impacts</u> for detailed methodology).

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

#### 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 6) and found to be:

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

### 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 7 and found to be:

- Farmed crustaceans—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 7.

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 <u>Appendix 3</u>.

The factors considered and the conclusions reached are presented below.

#### 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 following were considered:

- Head and shell removal 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.
- 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 head and shell off prawns as feed in research or public aquaria.
- Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal. 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 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 following were considered:

- Head and shell removal plus deveining is expected to reduce the amount of EHP present in the imported prawn and therefore the likelihood of entry of EHP.
- Head and shell removal plus deveining 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 head and shell off prawns as feed in research or public aquaria.
- Head and shell removal plus deveining is not expected to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. 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 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 following were considered:

- There are no reports on the effect of commercial cooking temperatures on EHP infectivity. 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). There are reports that spores of *E. cuniculi* and *E. hellem* heated to 70°C for 1 min resulted in 84% and 99% inhibition of growth, respectively (Li & Fayer 2006). However, exposure of the spores for 1 min at a lower temperature of 50°C only resulted in 74% (*E. cuniculi*) and 12% (*E. hellem*) inhibition of growth (Li & Fayer 2006). The sensitivity of microsporidian spores to temperature is further complicated as studies have shown that results can vary widely with other isolates and species of microsporidia and with other methods (Koudela, Kucerova & Hudcovic 1999; Li & Fayer 2006).
- Cooking is therefore not expected to reduce the likelihood of entry of EHP in imported prawns. This is because given the uncertainty regarding the effect of heat on EHP viability, it is assumed that cooking may only reduce the load of infectious EHP in imported prawn tissues, not completely inactivate it.
- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017, 2019). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed prawns being directly exposed to cooked prawns through the inlet channels is negligible.

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, applied was determined to be **negligible**.

#### 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 following were considered:

- Value-added products are products in which the uncooked prawns have had the head and shell removed and have been further processed (see <u>Value-added products</u>). The likelihood of entry of EHP 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 EHP in the product more than head and shell removal does.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.
- The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

Based on this information, the overall restricted risk of imported 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 2019i). 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 (OIE) (OIE 2020b) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). IMNV is exotic to Australia.

# **10.2** Technical information

The following technical information 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 2019i).

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 2019i).

# 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 OIE *Aquatic animal health code* (OIE Code) (OIE 2019b) include:

- *Penaeus esculentus* <sup>E</sup> (Gudkovs et al. 2015)
- Penaeus merguiensis <sup>E</sup> (Gudkovs et al. 2015)

• *Penaeus vannamei*<sup>N, E</sup> (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 <sup>N, E</sup> (NACA 2018; Srisala et al. 2020a; Tang et al. 2005; Tang et al. 2007c)
- *Penaeus stylirostris* <sup>E</sup> (Tang et al. 2005; Tang et al. 2007c).

IMNV-positive RT-PCR results have also been reported in the following species, however no active infection was demonstrated (E= experimental exposure):

- 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 2019i).

#### **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). More recently, IMNV has been reported from China (NACA & FAO 2015b), the Republic of Korea (NACA & FAO 2015a), Burma (NACA, OIE-RRAP & FAO 2016) and India (Sahul Hameed et al. 2017).

#### 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). In regions where IMNV is enzootic, prevalence may reach 100% (Andrade et al. 2007).

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 2019i).

#### 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). More recently, detection of IMNV by RT-PCR in grossly normal wild P. monodon of potential broodstock size caught from the Indian Ocean was 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). Assumedly, the farm had recent movement of wild *P. monodon* onto its premises, 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*.

#### 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 ( $\sim 1.0 \times 10^6$  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) and infectious hypodermal and haematopoietic necrosis virus (Feijó et al. 2013; 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 2019i; 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. 2007c).

# Tissue titre

Healthy *P. vannamei* injected with IMNV-infected tissue homogenate ( $\sim 1.0 \times 10^6$  IMNV viral copies) resulted in a viral load in the abdominal tissue that ranged from 45–2.27 × 10<sup>8</sup> 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<sup>3</sup>–5.10 × 10<sup>5</sup> copies/µg in the pleopods, 3.90 × 10<sup>3</sup>–8.15 × 10<sup>6</sup> copies/µg in gills, 3.09 × 10<sup>4</sup>–6.85 × 10<sup>8</sup> copies/µg in muscle and 1.33 × 10<sup>6</sup>–5.08 × 10<sup>8</sup> 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 2019i). 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 OIE *Manual of diagnostic tests for aquatic animals* (OIE Manual) provides details of the methods currently available for targeted surveillance and diagnosis of IMNV. The nested RT-PCR and qRT-PCR methods described in the OIE Manual are the recommended methods for targeted surveillance to declare freedom from IMNV (Andrade et al. 2007; OIE 2019i; Poulos & Lightner 2006).

The OIE Manual also describes the circumstances in which histopathology may be used to obtain a presumptive diagnosis of IMNV infection (OIE 2019i).

### 10.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments (OIE 2019i).

# 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 2019i). 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 2019i). 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 2019i). 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). More recently, 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 <u>General considerations and risk assessment process</u> and the technical information about IMNV presented in this chapter, the following 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 <u>Appendix 3</u>.

#### 10.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for IMNV.

- This draft 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 53–90% 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 2, the annual likelihood of entry of IMNV in imported prawns was estimated to be **high**.

#### **10.3.2** Exposure assessment

The following were considered relevant when conducting the exposure assessment for IMNV.

- 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 were considered unlikely to be directly exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent their introduction either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude IMNV or imported prawn wastes.
- 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.

Based on this information and using the qualitative likelihood descriptors in Table 2, the partial likelihood of exposure of each exposure group to IMNV in imported prawns was estimated to be:

- Farmed crustaceans—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 4 and was found to be:

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

#### 10.3.4 Consequence assessment

#### Partial likelihood of establishment and spread

The following were considered relevant when determining the partial likelihood of establishment and spread for IMNV.

- 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 highest for farmed crustaceans. This is due to the stressors 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 less 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.

Based on these considerations and using the descriptors in Table 2, the partial likelihood of establishment and spread of IMNV in each exposure group for the outbreak scenario (refer section 4.5.1 <u>Identification of the outbreak scenario</u>) was estimated to be:

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

#### Determining adverse impacts resulting from the outbreak scenario

The following were considered relevant when determining the adverse impacts resulting from establishment and spread of IMNV.

#### 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 OIE 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 restriction area were put in place for an outbreak of IMNV, there would be on-going 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 an OIE-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 restriction 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 12 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of IMNV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

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

Table 12 Overall impact of establishment and spread of IMNV for the outbreak scenario

#### 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 6) 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 7 and found to be:

- Farmed crustaceans—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 7.

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 <u>Appendix 3</u>.

The factors considered and the conclusions reached are presented below.

# 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 following were considered:

- 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.
- 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 head and shell off prawns as feed in research or public aquaria.
- Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal. 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 prawns with the biosecurity measure, head and shell removal, applied was determined to be **very low**.

# 10.4.2 Cooking

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

- IMNV is inactivated by heating at 60°C for at least 3 mins (OIE 2019i).
- Given the temperature required to inactivate IMNV is outside what would generally be expected for cooking prawns intended for human consumption, it is assumed that cooking may reduce, but not completely inactivate IMNV in imported prawn tissues and sufficient viable virus to cause disease will still be present. Therefore, cooking is not expected to reduce the likelihood of entry.
- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will also reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported

that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017, 2019). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed prawns being directly exposed to cooked prawns through the inlet channels is negligible.

#### Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, 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 following were considered:

- 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 <u>Value-added products</u>). 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.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.
- The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

Based on this information, the overall restricted risk of imported 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). Recently it was reported that LSNV and Wenzhou shrimp virus genotype 9 (WZSV9) are 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 (OIE) (OIE 2020b) and is not on the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2020b). MSGS is present on Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). LSNV (and WZSV9) are 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 following technical information 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 family *Luteoviridae* and an unassigned *Sobemovirus* (Sritunyalucksana et al. 2006a). It has recently 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* <sup>N</sup> (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* <sup>E</sup> (Anantasomboon et al. 2008a)
- *Penaeus indicus* <sup>E</sup> (Anantasomboon et al. 2008a)
- *Penaeus vannamei* <sup>E</sup> (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*<sup>N, E</sup> (Kumar et al. 2011; Panphut et al. 2011; Sritunyalucksana et al. 2006a)
- Penaeus semisulcatus N (Megahed 2019)
- *Penaeus vannamei*<sup>N, E</sup> (Kumar et al. 2011; Sakaew et al. 2008)

• *Scylla serrata* <sup>E</sup> (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 post-larvae 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* brooders (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), co-habitation 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<sup>7</sup> 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-exposure (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  $1.2 \times 10^6$  copies/µg of RNA,  $2.9 \times 10^5$  copies/µg in *P. vannamei*,  $4.7 \times 10^4$  copies/µg in *M. dobsoni* and  $8.2 \times 10^3$  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 by known agents while also complying with any 3 of the following clinical signs:

- i. unusually dark colour
- ii. average daily weight gain of less than 0.1 g/day at 4 months
- iii. unusually bright yellow markings
- iv. "bamboo-shaped" abdominal segments, and
- v. 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 LSNVpositive by RT-PCR (Kumar et al. 2011).

### Pathology

Retinopathy was observed exclusively in small LSNV-positive prawns collected from MSGSaffected 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 <u>General considerations and risk assessment process</u> and the technical information about LSNV presented in this chapter, the following 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 <u>Appendix 3</u>.

# 11.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for LSNV.

- 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) and shell 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 2, the annual likelihood of entry of LSNV in imported prawns was estimated to be **high**.

### 11.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for LSNV.

- 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 were considered unlikely to be directly exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent their introduction either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude LSNV or imported prawn wastes.
- 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. 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 2, the partial likelihood of exposure of each exposure group to LSNV in imported prawns was estimated to be:

- Farmed crustaceans—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 4 and was found to be:

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

# 11.3.4 Consequence assessment

### Partial likelihood of establishment and spread

The following were considered relevant when determining the partial likelihood of establishment and spread for LSNV.

- 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 greater for farmed and hatchery crustaceans than for wild crustaceans. This is due to the stressors 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 less, 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 2, 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 following were considered relevant when determining the adverse impacts resulting from establishment and spread of LSNV.

#### 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 OIE 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 restriction area were put in place for an outbreak of LSNV, there would be on-going 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 restriction 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 restriction 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 13 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of LSNV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

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	А
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	Е
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	Local	Minor	В
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	А
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	В

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

#### 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 6) 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 7 and found to be:

- Farmed crustaceans—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 7.

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 <u>Appendix 3</u>.

The factors considered and the conclusions reached are presented below.

# 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 following were considered:

- 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.
- 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 head and shell off prawns as feed in research or public aquaria.
- Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal. 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 prawns with the biosecurity measure, head and shell removal, applied was determined to be **very low**.

# 11.4.2 Cooking

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

- There are no reports of the effect of heating on LSNV infectivity.
- It is assumed that cooking may reduce, but not completely inactivate LSNV in imported prawn tissues and sufficient viable virus to cause disease will still be present. Therefore, cooking is not expected to reduce the likelihood of entry.
- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will also reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017, 2019). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed prawns being directly exposed to cooked prawns through the inlet channels is negligible.

#### Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, 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 following were considered:

- 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 <u>Value-added products</u>). 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.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.
- The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

Based on this information, the overall restricted risk of imported 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 2019j). TSV is 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 spread to the Americas, Asia and some parts in East Africa and the Middle East.

Taura syndrome is listed as a disease notifiable to the World Organisation for Animal Health (OIE) (OIE 2020b) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). TSV is exotic to Australia.

# **12.2** Technical information

The following technical information 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)
- 2) South-East Asian group (Nielsen et al. 2005; Wertheim et al. 2009)
- 3) Belize group (Erickson et al. 2005; Tang & Lightner 2005)
- 4) 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, 70°C for at least 30 mins or 90°C for at least 10 mins (Brock 1995; OIE 2019j).

# 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 *OIE Aquatic animal health code* (OIE Code) (OIE 2019b) 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 <sup>N, E</sup> (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* <sup>N, E</sup> (Lightner 1995).

Other host species shown to be susceptible to infection with TSV include (N= natural; E= experimental exposure):

- *Chelonibia patula* <sup>E</sup> (barnacle) (Overstreet, Jovonovich & Ma 2009)
- *Ergasilus manicatus* <sup>E</sup> (copepod) (Overstreet, Jovonovich & Ma 2009)
- Octolasmis muelleri E (barnacle) (Overstreet, Jovonovich & Ma 2009)
- *Penaeus chinensis* <sup>E</sup> (Lightner 1996b; Overstreet et al. 1997)
- *Penaeus merguiensis* <sup>E</sup> (Biosecurity Australia 2006; Ruangsri et al. 2005)
- *Penaeus monoceros* <sup>E</sup> (Ruangsri et al. 2005).

Also, TSV-positive RT-PCR and *in situ* hybridisation results have been reported in the following species (N= natural; E= experimental exposure), however no active infection has been demonstrated:

- *Callinectes sapidus* <sup>E</sup> (crab) (Erickson et al. 1997a)
- *Cherax quadricarinatus* <sup>E</sup> (crayfish) (Biosecurity Australia 2006)
- Cherax tenuimanus E (marron) (Biosecurity Australia 2006)
- *Fundulus grandis*<sup>E</sup> (Gulf killifish) (Overstreet, Jovonovich & Ma 2009)
- *Gallus gallus domesticus* <sup>E</sup> (chicken) (Garza et al. 1997; Vanpatten, Nunan & Lightner 2004)
- Larus atricilla N (sea gull) (Garza et al. 1997; Vanpatten, Nunan & Lightner 2004)
- *Macrobrachium lanchesteri* <sup>E</sup> (Kiatpathomchai et al. 2008)
- *Macrobrachium rosenbergii* <sup>N, E</sup> (Biosecurity Australia 2006; Nielsen et al. 2005)
- *Palaemon styliferus* <sup>E</sup> (Kiatpathomchai et al. 2008)
- *Penaeus duorarum* <sup>E</sup> (Brock 1997b; Overstreet et al. 1997)
- *Penaeus indicus* <sup>N</sup> (Tang et al. 2012)
- *Penaeus japonicus* N, E (Brock 1997b; Nielsen et al. 2005)
- *Penaeus schmitti* <sup>E</sup> (Brock 1997b)
- Sesarma mederi <sup>E</sup> (crab) (Kiatpathomchai et al. 2008)

- Scylla serrata <sup>E</sup> (crab) (Kiatpathomchai et al. 2008)
- *Trichocorixa reticulate* N (water boatman) (Lightner 1995)
- Uca vocans <sup>E</sup> (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 *P. merguiensis* became infected with a Thai isolate of TSV by injection but not following *per os* challenge (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: Burma (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 2019j).

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 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 co-habitation 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  $50\mu$ L of a TSV inoculum (Belize and Colombia TSV isolates) containing a total of  $2.5 \times 10^6$  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 x  $10^{6}$  TSV copies) and found 9.6 x  $10^{9}$ ,  $1.7 \times 10^{10}$ , and  $2.8 \times 10^{10}$  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; 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 2019j). 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 OIE *Manual of diagnostic tests for aquatic animals* (OIE Manual) (OIE 2019j) 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 (OIE 2019j). The OIE 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 (OIE 2019j).

# 12.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments (OIE 2019j).

# 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 2019j). 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 2019j).

# 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 <u>General considerations and risk assessment process</u> and the technical information about TSV presented in this chapter, the following 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 <u>Appendix 3</u>.

# 12.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for TSV.

- This draft risk review is generic and therefore the entry assessment assumes that TSV is present in all source countries.
- TSV infects various penaeid and 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 2, the annual likelihood of entry of TSV in imported prawns was estimated to be **high**.

# 12.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for TSV.

- 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 that are susceptible to TSV infection include, *P. monodon, P. merguiensis* and *M. rosenbergii*. Other TSV susceptible species and vectors, such as *C. quadricarinatus, P. indicus* and *P. japonicus* are widespread in Australian waters. The impact of TSV 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 were considered unlikely to be directly exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent their introduction either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude TSV or imported prawn wastes.
- 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 2, the partial likelihood of exposure of each exposure group to TSV in imported prawns was estimated to be:

- Farmed crustaceans—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 4 and was found to be:

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

# 12.3.4 Consequence assessment

# Partial likelihood of establishment and spread

The following were considered relevant when determining the partial likelihood of establishment and spread for TSV.

- 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*, *P. merguiensis* and *M. rosenbergii* are susceptible to TSV infection. Although susceptibility is TSV strain, species and exposure route dependent.
- Other crustacean species, such as *C. quadricarinatus*, *P. indicus* 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 greater for farmed and hatchery crustaceans than for wild crustaceans. This is due to the stressors 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 less 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.
- 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 2, the partial likelihood of establishment and spread of TSV in each exposure group for the outbreak scenario (refer section 4.5.1 <u>Identification of the outbreak scenario</u>) was estimated to be:

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

#### Determining adverse impacts resulting from the outbreak scenario

The following were considered relevant when determining the adverse impacts resulting from establishment and spread of TSV.

#### 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 OIE 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 restriction 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 restriction 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 an OIE-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 restriction 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 14 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of TSV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

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	В
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	Е
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	District or region	Minor	С
	Environment (biodiversity, endangered species and the integrity of ecosystems)	National	Minor	Е
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	В

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

# 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 6) 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 7 and found to be:

- Farmed crustaceans—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 7.

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 <u>Appendix 3</u>.

The factors considered and the conclusions reached are presented below.

# 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 following were considered:

- 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.
- 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 head and shell off prawns as feed in research or public aquaria.

• Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal. 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 prawns with the biosecurity measure, head and shell removal, applied was determined to be **very low**.

# 12.4.2 Cooking

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

- TSV is inactivated by heating at 70°C for at least 30 mins or 90°C for at least 10 mins (OIE 2019j).
- Given the temperature required to inactivate TSV is outside what would generally be expected for cooking prawns intended for human consumption, it is assumed that cooking may reduce, but not completely inactivate TSV in imported prawn tissues and sufficient viable virus to cause disease will still be present. Therefore, cooking is not expected to reduce the likelihood of entry.
- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will also reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017, 2019). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed prawns being directly exposed to cooked prawns through the inlet channels is negligible.

# Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, 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 following were considered:

- 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 <u>Value-added products</u>). 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.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less

attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.

• The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

Based on this information, the overall restricted risk of imported 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 2019a). AHPND is characterised by sudden mass mortalities that commonly occur within 30–35 days of stocking grow-out ponds with postlarvae or juveniles (OIE 2019a).

Vp AHPND carries a plasmid with genes that encodes two Pir-like toxins (*Photorhabdus* insectrelated), PirA and PirB (OIE 2019a). 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 OIE *Aquatic animal health code* (OIE Code) Article 9.1.1 (OIE 2019b) and this definition is used for the purposes of this draft risk review.

For the purposes of the OIE 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.

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 in 2009 and has since spread to several Asian countries and other parts of the world (Flegel 2012; Lightner et al. 2012a; OIE 2019a).

AHPND is listed as a disease notifiable to the World Organisation for Animal Health (OIE) (OIE 2020b) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). Australia has reported hepatopancreatitis in prawns to the OIE but it did not satisfy the OIE case definition of AHPND as it was caused by a *Vibrio* species other than Vp AHPND (as identified using whole genome sequencing) (OIE 2016). AHPND is exotic to Australia.

# 13.2 Technical information

The following technical information 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 the 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,  $\sim$ 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 recently. 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 2019a; 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).

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).

*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 OIE Code (OIE 2019b) include:

- *Penaeus monodon* <sup>N</sup> (de la Peña et al. 2015; Eshik et al. 2018; Lightner et al. 2012a)
- Penaeus vannamei <sup>N, E</sup> (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 <sup>E</sup> (Kumar et al. 2018; Kumar et al. 2019; Muthukrishnan et al. 2019)
- *Exopalaemon carinicauda* <sup>E</sup> (Ge et al. 2018; Ge et al. 2017)
- Macrobrachium rosenbergii N, E (Kumar et al. 2018; Tun et al. 2017)
- Penaeus chinensis (OIE 2019a)
- Penaeus japonicus (OIE 2019a)
- Penaeus semisulcatus <sup>N, E</sup> (Megahed 2018).

Species for which Vp AHPND-positive PCR results have also been reported include (N= natural exposure):

- Bait worms (polychaetes) <sup>N</sup> (FAO 2017; Thitamadee et al. 2016; Tran 2018)
- Bivalves (oysters, clams) <sup>N</sup> (Thitamadee et al. 2016).

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 2019a; Soto-Rodriguez et al. 2015; Tran et al. 2013b).

# **Geographical distribution**

AHPND was first reported in China in 2009 (Flegel 2012; Lightner et al. 2012a) and later in: Bangladesh (Eshik et al. 2018), Burma (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 2019d), Thailand (Flegel 2012; Joshi et al. 2014b; Kondo et al. 2014) and Vietnam (Kondo et al. 2015; Tran et al. 2013b).

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 2019a; 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 more recent 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).

Higher prevalence of AHPND have been reported in farmed *P. monodon* compared to farmed *P. vannamei* (Boonyawiat 2017; FAO 2017; Songsangjinda 2017). Vp AHPND has been detected in live broodstock feeds such as polychaetes and bivalves (FAO 2017; Thitamadee et al. 2016; Tran 2018).

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 2019a). 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. 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 2019a).

Transmission via live feeds (clams, oysters, polychaetes, and fresh squid meat) has been suggested following the finding of Vp AHPND-positive PCR results from live polychaete 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). More recently, 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 AHPNDcausing 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 recent 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 been 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<sup>7</sup> Vp AHPND colony-forming units/ml (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<sup>6</sup>–10<sup>8</sup> 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<sup>4</sup> 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 2019a). 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 2019a).

*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<sup>4</sup> 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).

#### Tissue tropism

Vp AHPND is reported to target gut-associated tissues and organs including hepatopancreas, stomach, midgut and hindgut (Lightner et al. 2012a; OIE 2019a).

Vp AHPND releases PirA and PirB toxins into the stomach of the prawn, which causes sloughing of epithelial cells into the hepatopancreatic tubules (Lai et al. 2015; 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). With the progress of the disease, the hepatopancreas can be atrophied to a degree that causes the death of the affected prawn (Lai et al. 2015; Soto-Rodriguez et al. 2015).

#### 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  $2.5 \times 10^3$ – $3.4 \times 10^3$  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 2019a). 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). Identification of *V. parahaemolyticus* can be achieved using 16S rRNA PCR (Weisburg et al. 1991) or toxR-targeted PCR (Kim et al. 1999) and sequencing. PCR solely targeting 16S rRNA gene has been reported to be insufficient to identify *Vibrio* to species level (Muthukrishnan et al. 2019).

Chapter 2.2.1 of the OIE *Manual of diagnostic tests for aquatic animals* (OIE 2019m) 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; OIE 2019a).

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 2019a).

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 2019a; 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).

No AHPND-resistant domesticated stocks of penaeid prawns have been developed (OIE 2019a). However, genetic lines with expected small to moderate advances in the population by selection for the resistance indicators for AHPND have been reported recently (Campos-Montes et al. 2020). Immersion challenge of three Latin American prawn lines with  $1 \times 10^5$  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 Caro et al. 2020). *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).

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). On this basis, 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 <u>General considerations and risk assessment process</u> and the technical information about Vp AHPND presented in this chapter, the following risk assessment was completed.

A summary of the risk assessment values for determining if Vp AHPND meets Australia's ALOP are shown in <u>Appendix 3</u>.

#### 13.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for Vp AHPND.

- This draft 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 2, the annual likelihood of entry of Vp AHPND in imported prawns was estimated to be **very low**.

#### 13.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for Vp AHPND.

- 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.
- 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 were considered unlikely to be exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent the introduction of imported prawns either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude Vp AHPND or imported prawn wastes.
- 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. The host range for Vp APPND 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 2, 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 4 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 following were considered relevant when determining the partial likelihood of establishment and spread for Vp AHPND.

- 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 greater for farmed and hatchery crustaceans than for wild crustaceans. This is due to the stressors 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 less 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 2, the partial likelihood of establishment and spread of Vp AHPND in each exposure group for the outbreak scenario (refer section 4.5.1 <u>Identification of the outbreak scenario</u>) was estimated to be:

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

#### Determining adverse impacts resulting from the outbreak scenario

The following were considered relevant when determining the adverse impacts resulting from establishment and spread of Vp AHPND.

#### 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 OIE and is included on Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). 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 restriction area were put in place for an outbreak of Vp AHPND, there would be on-going 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 which may be indirectly affected by movement restriction areas that encompass potential susceptible species and vectors.
- 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 an OIE-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

- No endangered Australian crustacean species, or closely related species, are susceptible to Vp AHPND.
- The impacts of Vp AHPND 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 restriction 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 15 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of Vp AHPND. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

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	А
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	Е
	Economic (international trade effects)	District or region	Minor	С
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	А
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	В

Table 15 Overall impact of establishment and spread of Vp AHPND for the outbreak scenario

#### 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 6) 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 7 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 7.

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 <u>Appendix 3.</u>

The factors considered and the conclusions reached are presented below.

#### 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 following were considered:

- 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.
- 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 head and shell off prawns as feed in research or public aquaria.

- Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. However, given the susceptibility of Vp AHPND to freezing and thawing it is considered that there is a reduction in the amount of viable Vp AHPND that would be contained in the prawns. In the case of farmed and hatchery crustaceans it is considered that the decrease in likelihood of use as feed and the reduction in viable agent decreases the exposure likelihood to negligible levels. 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.
- Risk is not eliminated in the wild due to the ability of Vp AHPND to be a free-living organism in the marine environment.

#### Conclusion

Based on this information, the overall restricted risk of imported 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 following were considered:

- *V. parahaemolyticus* in seafood is sensitive to heating. No reported investigations specific to the stability of Vp AHPND to heat treatments were found. It is assumed that cooking would decrease the load of infectious Vp AHPND on entry.
- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will also reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed prawns being directly exposed to cooked prawns through the inlet channels is negligible.

#### Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, 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 following were considered:

- 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 <u>Value-added products</u>). The likelihood of entry of Vp AHPND 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 Vp AHPND in the product more than head and shell removal does.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional

benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.

• The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

Based on this information, the overall restricted risk of imported uncooked 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 2019k). 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 2019k; 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 (OIE) (OIE 2020b) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). 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).

## 14.2 Technical information

The following technical information 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. 2020; 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  $-40^{\circ}$ 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-exposure 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 OIE, 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 more recent study found that prawns which were fed tissue from WSSVpositive 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. 2020).

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 2019k; 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 then spread 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, an outbreak of WSD in prawn farms on the Logan River in South-East Queensland was confirmed and reported on 1 December 2016 (Department of Agriculture and Water Resources 2017c). WSD is limited to an area in South-East Queensland and is under an official control program (Department of Agriculture and Fisheries 2019).

#### Prevalence

Prevalence of WSSV of up to 100% have been reported in farmed prawn populations (OIE 2019k). 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 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. 2020b). 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. 2020b). 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).

#### 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, polychaete worms, 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).

Recently, 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. Polychaete worms, 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 WSSVinfected 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 promptly 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).

On 1 December 2016, it was confirmed that WSD was present in Australia on a prawn farm on the Logan River in South-East Queensland. An emergency response was initiated to contain the disease and eradicate it. 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.

To date, the origin of the outbreak has not been determined (Department of Agriculture and Water Resources 2017c). In early-2020, WSSV was again detected in 2 prawn farms on the Logan River in South-East Queensland that had been previously affected with WSD during the 2016–17 outbreak. Genetic evidence indicated that this was not a new incursion as the virus strain detected is the same one that caused the original 2016–17 outbreak.

#### 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<sup>4</sup> 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  $5 \times 10^6$  WSSV genome copies into *P. vannamei* resulted in 100% mortality within 5 days postinfection. In an infection study of *P. monodon*, intramuscular injection (0.1ml) of WSSV stock at  $2.62 \times 10^6$  genome copies/µl was sufficient to result in moribund prawns within 3 days postinfection (Gomathi, Otta & Shekhar 2015). The WSSV LD<sub>50</sub> (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<sub>50</sub> 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  $1 \times 10^5$  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 107.53 times more WSSV was needed to infect a prawn via gavage and 10<sup>8.03</sup> 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 glad are the main target organs for WSV 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  $1.6 \times 10^9$  and  $3 \times 10^{10}$  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  $2.55 \times 10^9$  in haemolymph,  $1.6 \times 10^9$  in pleopods,  $1.2 \times 10^9$  in gills,  $1.9 \times 10^8$  in muscle and  $9 \times 10^7$  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 ( $2 \times 10^{10}$  WSSV copies/g of tissue) than the tail ( $1.53 \times 10^{10}$  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  $1.26 \times 10^{11}$  WSSV copies in the head and  $1.33 \times 10^{11}$  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 ( $7.32 \times 10^{10}$  WSSV copies/g of tissue) was about 4 times higher than that present in the tail muscle ( $1.86 \times 10^{10}$  WSSV copes/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  $6.3 \times 10^6$  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  $1.0 \times 10^8$  genome copies/µg of DNA in the gills and stomach and  $1.0 \times 10^7$  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  $3.0 \times 10^7$  in the gills,  $2.7 \times 10^7$  in the gut,  $1.5 \times 10^7$  in muscle,  $3.3 \times 10^6$  in the haemolymph,  $2.9 \times 10^6$  in the eyestalk,  $2.5 \times 10^6$  in the pleopod and  $2.4 \times 10^6$  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 found to be  $3.8 \times 10^9$  in sub-cuticular epithelium,  $3.8 \times 10^7$  in pleopod and  $3.81 \times 10^6$  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  $2.10 \times 10^6$  genome copies/µg DNA (Durand & Lightner 2002). Jang et al. (2009) reported a mean WSSV viral load of  $1.5 \times 10^4$  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 2019k; 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 2019k). 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 2019k).

#### 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 *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 (OIE 2019k).

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 2019k).

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).

#### 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 \$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).

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).

Although WSSV has been detected in wild crustacean populations (Hood et al. 2019; Muhammad et al. 2020; Orosco & Lluisma 2017; Saravanan et al. 2017), no reports were found about the impact of WSSV on wild crustacean populations.

## 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 and eradication program. WSSV is capable of causing adverse effects. Based on the preceding information risk assessment is warranted.

## 14.3 Risk assessment

Based on chapter 4 <u>General considerations and risk assessment process</u> and the technical information about WSSV presented in this chapter, the following 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 <u>Appendix 3</u>.

#### 14.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for WSSV.

- This draft 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 2, the annual likelihood of entry of WSSV in imported prawns was estimated to be **high**.

#### 14.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for WSSV.

- 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 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 were considered unlikely to be directly exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent their introduction either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude WSSV or imported prawn wastes.
- 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 2, the partial likelihood of exposure of each exposure group to WSSV in imported prawns was estimated to be:

- Farmed crustaceans—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 4 and was found to be:

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

#### 14.3.4 Consequence assessment

#### Partial likelihood of establishment and spread

The following were considered relevant when determining the partial likelihood of establishment and spread for WSSV.

- 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, polychaete worms, 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 the stressors 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 2, the partial likelihood of establishment and spread of WSSV in each exposure group for the outbreak scenario (refer section 4.5.1 <u>Identification of the outbreak scenario</u>) was estimated to be:

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

#### Determining adverse impacts resulting from the outbreak scenario

The following were considered relevant when determining the adverse impacts resulting from establishment and spread of WSSV.

#### 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, the Logan River prawn farming industry production losses in 2016–17 were estimated to be approximately \$23.5 million (excluding their response costs) and it was estimated that the cost of lost hatchery and breeding stocks were \$5–6 million.
- 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). 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 2019k). 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 OIE 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 WSSV 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 WSSV outbreak are significant. When the WSSV outbreak in the prawn farms on the Logan River, Queensland occurred, the Commonwealth and Queensland governments spent more than AU\$47 million in response, eradication and control programs. Due to the on-going 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 which may be indirectly affected by movement restriction areas that encompass all species potentially capable of transmitting the virus. In Australia, during the 2016–17 WSSV outbreak, the impact of the movement restriction area on the fisheries industry was estimated to be \$20.5 million. As the movement restriction area remains in place these costs are on-going.
- 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 an OIE-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 2019k). 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 restriction put in place due to an outbreak of WSSV which may impact on social amenity.
- 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 16 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of WSSV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

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	Е
	Economic (domestic trade effects and impact on other associated industries)	National	Minor	Е
	Economic (international trade effects)	District or region	Minor	С
	Environment (biodiversity, endangered species and the integrity of ecosystems)	National	Minor	Е
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	С

Table 16 Overall impact of establishment and spread of WSSV for the outbreak scenario

#### Conclusion

The overall impact of establishment and spread of 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 6) 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 7 and found to be:

- Farmed crustaceans—Moderate.
- 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 7.

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 <u>Appendix 3</u>.

The factors considered and the conclusions reached are presented below.

#### 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 following were considered:

- 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<sup>10</sup> 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.
- 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 head and shell off prawns as feed in research or public aquaria.
- Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal. 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 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 following were considered:

- 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.
- Head and shell removal plus deveining 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. This reduction is not expected to be more than the reduction seen with head and shell removal only.
- Head and shell removal plus deveining is not expected to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. 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 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 testing

When determining if head and shell removal combined with pre-export testing would reduce the overall risk of WSSV to meet Australia's ALOP, the following were considered:

- There are sensitive qPCR methods available to detect WSSV in prawns (OIE 2019k).
- Post-processing batch testing of prawns for WSSV prior to export would reduce the likelihood of entry. Under this scenario it is assumed that the testing is not conducted under a department approved testing or sampling system.
- The application of pre-export testing in combination with head and shell removal would not reduce exposure likelihoods more than the reduction due to head and shell removal only. This is because pre-export testing does not physically change the prawn in a manner that reduces the likelihood of them being used for unintended purposes (such as bait or berley).

#### Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, head and shell removal in combination with pre-export 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 testing

When determining if head and shell removal combined with pre-export and on-arrival testing would reduce the overall risk of WSSV to meet Australia's ALOP, the following were considered:

- There are sensitive qPCR methods available to detect WSSV in prawns (OIE 2019k).
- Batch testing of prawns for WSSV on-arrival in Australia would reduce the likelihood of entry. Under this scenario, testing and sampling is being conducted under departmental control and oversight.
- The application of on-arrival and pre-export testing in combination with head and shell removal would not reduce exposure likelihoods more than the reduction due to head and shell removal only. This is because pre-export and on-arrival testing does not physically change the prawn in a manner that reduces the likelihood of them being used for unintended purposes (such as bait or berley).

#### Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, head and shell removal in combination with pre-export and on-arrival 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 following were considered:

- Heat treatment has been shown to inactivate WSSV suspended in sterile water at 55°C for 90 mins, 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 1 min, 70°C for 0.2 min in tissue homogenates (Nakano et al. 1998), and at 60°C for 20 mins for homogenised viral preparations (Balasubramanian et al. 2006). 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). WSSV-positive prawns boiled at 100°C for 1, 3, 5, 10 and 30 mins were not capable of transmitting WSSV to prawns following *per os* exposure (Aranguren et al. 2020). In these experiments, it took approximately 48 seconds for the core temperature of the prawns to reach 70°C (Aranguren et al. 2020).
- The reported time and temperatures required to inactivate WSSV are within the parameters generally expected for cooking prawns intended for human consumption. Therefore, it is assumed that cooking would reduce the load of infectious WSSV in imported prawn tissues but not completely inactivate it. It is assumed that some infectious virus may remain, but that cooking will significantly reduce the likelihood of entry.
- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will also reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed crustaceans being directly exposed to cooked prawns through the inlet channels is negligible.

#### Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, 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 following were considered:

- 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 <u>Value-added products</u>). 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.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.
- The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

Based on this information, the overall restricted risk of imported 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 2019]; 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 is restricted to 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 a recent report of its presence in the Republic of Korea (Kim et al. 2020).

Infection with YHV1 is listed as a disease notifiable to the World Organisation for Animal Health (OIE) (OIE 2020b) and is on Australia's *National list of reportable diseases of aquatic animals* (AHC 2018). Infection with YHV8 is not notifiable to the OIE (OIE 2020b), is not on Australia's *National list of reportable diseases of aquatic animals* (AHC 2018) and is not in the *List of diseases in the Asia-Pacific* (NACA, OIE-RRAP & FAO 2020b). YHV genotypes 2, 6 and 7 are present in Australia (Cowley et al. 2000; FRDC 2018; Mohr et al. 2015), however YHV1 and YHV8 are exotic.

The only members of the yellow head complex which comply with the criteria described in the OIE *Aquatic animal health code* (OIE Code) Article 2.1.2 *Hazard Identification* (OIE 2019b) and have been retained for risk assessment, are YHV1 and YHV8. However, this risk assessment 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 following technical information 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).

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 inactivated by heating at 60°C for 15–30 mins (Cowley et al. 2011; Flegel et al. 1995).

# 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 OIE Code (OIE 2019b) include:

- *Metapenaeus affinis* <sup>E</sup> (prawn) (Longyant et al. 2006)
- Palaemonetes pugio E (prawn) (Ma, Overstreet & Jovonovich 2009)
- *Penaeus monodon*<sup>N, E</sup> (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993)
- *Penaeus stylirostris* <sup>N, E</sup> (Castro-Longoria et al. 2008; de la Rosa-Velez et al. 2006; Lightner 1996b; Lu et al. 1994)
- Penaeus vannamei <sup>N, E</sup> (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* <sup>E</sup> (Longyant et al. 2005)
- *Metapenaeus bennettae* <sup>E</sup> (prawn) (OIE 2010)
- *Metapenaeus brevicornis* <sup>E</sup> (Longyant et al. 2006)
- *Metapenaeus ensis* <sup>E</sup> (Chantanachookin et al. 1993; Flegel et al. 1995)
- Palaemon serrifer E (Longyant et al. 2005)
- *Palaemon styliferus* <sup>E</sup> (Flegel et al. 1995; Longyant et al. 2005)
- *Penaeus aztecus* <sup>E</sup> (Lightner 1996b; Lightner et al. 1998)
- *Penaeus duorarum* <sup>E</sup> (Lightner 1996b; Lightner et al. 1998)
- *Penaeus japonicus* <sup>N</sup> (Wang et al. 1996)
- *Penaeus merguiensis* <sup>E</sup> (Boonyaratpalin et al. 1993; Flegel et al. 1995)
- *Penaeus setiferus* <sup>E</sup> (Lightner 1996b; Lightner et al. 1998).

YHV1-positive RT-PCR results have also been reported in the following species (N=natural; E=experimental exposure), however no active infection has been demonstrated:

- *Acetes* sp. N (paste shrimp) (Chantanachookin et al. 1993; Flegel, Fegan & Sriurairatana 1995; Flegel et al. 1995)
- *Callinectes sapidus* <sup>E</sup> (blue crab) (Overstreet, Jovonovich & Ma 2009)
- *Chelonibia patula* <sup>E</sup> (acorn barnacle) (Overstreet, Jovonovich & Ma 2009)
- *Cherax quadricarinatus* <sup>E</sup> (Soowannayan et al. 2015)
- *Ergasilus manicatus* <sup>E</sup> (cyclopoid copepod) (Overstreet, Jovonovich & Ma 2009)
- *Fundulus grandis* <sup>E</sup> (Gulf killifish) (Overstreet, Jovonovich & Ma 2009)
- *Octolasmis muelleri* <sup>E</sup> (gooseneck barnacle) (Overstreet, Jovonovich & Ma 2009)
- *Portunus pelagicus* <sup>E</sup> (crab) (Boonsaeng et al. 2000)
- *Scylla serrata* <sup>E</sup> (crab) (Boonsaeng et al. 2000)
- Sesarma sp E (crab) (Boonsaeng et al. 2000)
- *Uca spinata* <sup>E</sup> (crab) (Boonsaeng et al. 2000).

Species considered susceptible to infection (N= natural exposure) with YHV8 include:

- *Macrobrachium rosenbergii* <sup>№</sup> (Zhu et al. 2016)
- Penaeus chinensis N (Zhu et al. 2016)
- *Penaeus japonicus*<sup>N</sup> (Zhu et al. 2016)
- Penaeus vannamei N (Zhu et al. 2016).

YHV1 affects late postlarval stages, juvenile and adult prawns (OIE 2019l). *P. monodon* are susceptible to YHV1 beyond postlarvae 15 (OIE 2019l). 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) 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 recently 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 2019l; 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 2019l). 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 2019l).

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 (within 3–5 days of the first appearance of clinical signs) during disease outbreaks (OIE 2019l). 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). In both natural infections and challenge trials, cumulative mortalities up to 100% within 3–5 days post-infection were reported due to YHD (Boonyaratpalin 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 co-habitation of naïve prawns with infected prawns (Flegel et al. 1995; Lightner 1996b). *P. monodon* exposed to YHV1 by co-habitation with experimentally infected crabs or infected red claw crayfish also contracted 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 2019).

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 co-habitation with infected prawns were shown to be infected by YHV1 by RT-PCR and by transmission bioassays, however they showed no 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* sp.) 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  $3.8 \times 10^{10}$ – $1.5 \times 10^{11}$  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<sup>6</sup> YHV viral copies/g of prawn resulted in 100% mortality of prawns within 48 hours. *P. monodon* injected with approximately 10<sup>6</sup> 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),  $5 \times 10^5$  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<sup>6</sup> 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 2019l).

#### 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 OIE *Manual of diagnostic tests for aquatic animals* (OIE 2019l) 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 (OIE 2019I).

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; OIE 2019l). 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 (OIE 2019l).

# 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. 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. 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 <u>General considerations and risk assessment process</u> and the technical information about YHV1 presented in this chapter, the following 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 <u>Appendix 3</u>.

#### 15.3.1 Entry assessment

The following were considered relevant when conducting the entry assessment for YHV1.

- 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.

Based on this information and using the qualitative likelihood descriptors in Table 2, the annual likelihood of entry of YHV1 in imported prawns was estimated to be **high**.

#### 15.3.2 Exposure assessment

The following were considered relevant when conducting the exposure assessment for YHV1.

- 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 were considered unlikely to be directly exposed to imported prawns (or associated wastes) because on-farm biosecurity measures should prevent their introduction either intentionally (for example, for feed) or unintentionally (through direct entry via the water inlet channels). However, not all farms have implemented standards of entry-level biosecurity for intake water that would exclude YHV1 or imported prawn wastes.
- 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.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 2, the partial likelihood of exposure of each exposure group to YHV1 in imported prawns was estimated to be:

- Farmed crustaceans—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 4 and was found to be:

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

#### 15.3.4 Consequence assessment

#### Partial likelihood of establishment and spread (PLES)

The following were considered relevant when determining the partial likelihood of establishment and spread for YHV1.

- YHV1 can be transmitted by ingestion of infected tissues, co-habitation 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.
- Other YHV1 susceptible species are widespread in Australian waters, including *C. quadricarinatus.*
- 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 the stressors 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 less 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 2, 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 following were considered relevant when determining the adverse impacts resulting from establishment and spread of YHV1.

#### 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 OIE 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 restriction area were put in place for an outbreak of YHV1, there would be on-going 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 including species which may be indirectly affected by movement restriction areas that encompass all potential susceptible species.
- 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 an OIE-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.
- *C. tenuimanus* is listed as critically endangered. If YHV1 were able to cause clinical disease in *C. tenuimanus* it could result in a significant impact on the survival of that already endangered species. It is unknown if *C. tenuimanus* may be affected by YHV1.
- 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 restrictions 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 17 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of YHV1. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section 4.5.6 <u>Determining impacts</u> for detailed methodology).

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	В
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	Е
	Economic (domestic trade effects and impact on other associated industries)	National	Minor	Е

Table 17 Overall impact of establishment and spread of YHV1 for the outbreak scenario

Effects	Criteria	Level	Impact	Score
	Economic (international trade effects)	District or region	Minor	С
	Environment (biodiversity, endangered species and the integrity of ecosystems)	National	Minor	Е
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	С

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 6) 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 7 and found to be:

- Farmed crustaceans—Moderate.
- 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 7.

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 <u>Appendix 3</u>.

The factors considered and the conclusions reached are presented below.

#### 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 following were considered:

- 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.
- Head and shell removal is not expected to significantly reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. Therefore, there is no reduction in the likelihood of exposure of wild crustaceans to imported prawns due to head and shell removal. Additionally, the very small likelihood for farmed crustaceans to be directly exposed to imported prawns used as bait in farm inlet channels remains.

Based on this information, the overall restricted risk of imported 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 following were considered:

- 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.
- Head and shell removal plus deveining 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. This reduction is not expected to be more than the reduction seen with head and shell removal only.
- Head and shell removal plus deveining is not expected to reduce the likelihood of imported prawns being used by recreational fishers as bait or berley. 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 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 testing

When determining if head and shell removal combined with pre-export testing would reduce the overall risk of YHV1 to meet Australia's ALOP, the following were considered:

- 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 would reduce the likelihood of entry. Under this scenario it is assumed that the testing is not conducted under a department approved testing or sampling system.
- The application of pre-export testing in combination with head and shell removal would not reduce exposure likelihoods more than the reduction due to head and shell removal only. This is because pre-export testing does not physically change the prawn in a manner that reduces the likelihood of them being used for unintended purposes (such as bait or berley).

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, head and shell removal combined with pre-export 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 testing

When determining if head and shell removal and testing combined with pre-export and onarrival testing would reduce the overall risk of YHV1 to meet Australia's ALOP, the following were considered:

- 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 would reduce the likelihood of entry. Under this scenario, testing and sampling is conducted under departmental control and oversight.
- The application of on-arrival and pre-export testing in combination with head and shell removal would not reduce exposure likelihoods more than the reduction due to head and shell removal-only. This is because pre-export and on-arrival testing does not physically change the prawn in a manner that reduces the likelihood of them being used for unintended purposes (such as bait or berley).

#### Conclusion

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, head and shell removal in combination with pre-export and on-arrival testing, applied was determined to be **very low.** 

#### 15.4.5 Cooking

When determining if cooking would reduce the overall risk of YHV1 to meet Australia's ALOP, the following were considered:

- YHV1 is inactivated by heating at 60°C for 15–30 mins (Cowley et al. 2011; Flegel et al. 1995).
- Given the temperature required to inactivate YHV1 is outside what would generally be expected for cooking prawns intended for human consumption, it is assumed that cooking may reduce, but not completely inactivate YHV1 in imported prawn tissues and sufficient viable virus to cause disease will still be present. Therefore, cooking is not expected to reduce the likelihood of entry.

- The likelihood of farmed and hatchery crustaceans being deliberately exposed to cooked prawns is significantly reduced. This is because cooked prawns would be unattractive feed for the maturation of broodstock or for crustaceans in research and public aquaria as the nutritional benefits are removed through cooking.
- Cooking will also reduce the likelihood of prawns being used as bait or berley and therefore exposure of wild crustaceans to imported prawns. However, this reduction would be less than the expected reductions for farmed and hatchery crustaceans as it has been reported that there is a small amount of use of cooked prawns as bait or berley by recreational fishers (Kantar Public 2017). There would also be a reduction in the likelihood of cooked prawns being used as bait or berley in prawn inlet channels. It is considered that the overall likelihood of farmed prawns being directly exposed to cooked prawns through the inlet channels is negligible.

Based on this information, the overall restricted risk of imported prawns with the biosecurity measure, cooking, applied was determined to be **very low**.

#### 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 following were considered:

- 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 <u>Value-added products</u>). 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.
- The likelihood of exposure of farmed and hatchery crustaceans to VAP is significantly reduced because VAP are unlikely to be used as feed as they would lack the nutritional benefits of whole, uncooked prawns. There would also be a reduction in the likelihood of VAP being used as bait or berley in prawn inlet channels because VAP would be less attractive to use as bait or berley compared to unprocessed (whole or head and shell off), uncooked prawns.
- The likelihood of wild crustaceans being exposed to VAP would be significantly reduced because 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.

#### Conclusion

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

# 16 Proposed biosecurity measures for imported prawns

The following provides the proposed import conditions for prawns and prawn products exported to Australia. Details of the risk assessment values for determining how the below biosecurity measures manage the biosecurity risk for each hazard to a level that meets Australia's appropriate level of protection (ALOP) are shown in <u>Appendix 3</u>.

Those seeking to propose alternative biosecurity measures 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, were not considered in detail for each hazard as part of this draft risk review.

# 16.1 Documentation

The importer must obtain a permit to import all uncooked prawns, breaded, battered or crumbed prawns and dumpling and dim sum-type products containing uncooked prawns into Australia for human consumption from the Department of Agriculture, Water and the Environment 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 the above information as well as any other criteria deemed relevant by the Delegate of the Director of Biosecurity.

Cooked prawns and prawn products do not require an import permit but will be required to meet conditions that are specified in the <u>Biosecurity (Prohibited and Conditionally Non-prohibited Goods) Determination 2016</u>. These conditions specify that the cooked prawns are accompanied by a certificate from a body listed in the <u>List of Overseas Authorities—Aquatic Animals for Import</u> (also known as the 'competent authority' (CA)).

# 16.2 Certification

Prawns may be imported into Australia under the following conditions.

# 16.2.1 Prawns sourced from a country, zone or compartment that is recognised by Australia to be free of pathogenic agents of biosecurity concern

Prawns sourced from disease free countries, zones or compartments may be exported to Australia as 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.

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) have been sourced from a country, compartment or zone that is recognised by Australia to be free of all the following pathogenic agents:
  - i. "Candidatus Hepatobacter penaei" (only if the product is chilled)

- ii. covert mortality nodavirus
- iii. Enterocytozoon hepatopenaei
- iv. infectious myonecrosis virus
- v. Laem-Singh virus
- vi. Taura syndrome virus
- vii. Vibrio parahaemolyticus strains containing Pir toxins
- viii. white spot syndrome virus (WSSV)
- ix. 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) each package is marked with the words "For human consumption only. Not to be used as bait or feed for aquatic animals".

If uncooked prawns are sourced from a country, zone or compartment recognised by Australia to be free of the above pathogenic agents, batch-testing for WSSV and YHV1 pre-export and onarrival 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 uncooked prawns 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 above.

#### 16.2.2 Uncooked prawns

Uncooked prawns are prawns which have been deveined and had the head and shell removed (the last shell segment and tail fans permitted) and 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.

All imported uncooked prawns must be free from both WSSV and YHV1.

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) product from each batch (see <u>Appendix 4</u> for batch definition) has been found postprocessing to be free of WSSV and YHV1 based on a sampling and testing method recognised by the World Organisation for Animal Health (OIE) for demonstrating absence of disease
- 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 in Australia each batch of uncooked prawns will be subject to seals intact inspection and testing for WSSV and YHV1 at a screening laboratory approved by the department.

# 16.2.3 Breaded, battered and crumbed prawns

Breaded, battered and crumbed (BBC) prawns are 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.

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.

The CA in the exporting country must certify on an official government health certificate that:

- a) the BBC prawns have been processed, inspected and graded in premises approved by and under the control of the CA
- b) the prawns are free from visible signs of infectious diseases prior to coating
- c) the BBC prawns have undergone a par-cooking step (for example, pre-frying<sup>1</sup> or baking) after the prawns have been coated to solidify and adhere the coating to the prawn.

Prawn products that do not meet all the import conditions outlined for BBC prawns will be subject to the import conditions for '<u>Uncooked prawns'</u>.

# 16.2.4 Dumpling and dim sum-type products which contain uncooked prawns

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. They include all types of dumpling, spring roll, samosa, roll, ball or dim sum-type products (containing uncooked prawns).

The CA in the exporting country must certify on an official government health certificate that the dumpling and dim sum-type products:

- a) 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.

# 16.2.5 Cooked prawns

Minimum cooking times and temperatures are not specified for cooked prawns. However, the CA must be able to certify that all the protein in the prawn meat has coagulated and no raw prawn meat remains. An example of a cooking time considered necessary to achieve coagulation of

<sup>&</sup>lt;sup>1</sup> 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, <u>Code of practice for fish and fishery</u> <u>products, CAC/RCP 52-2003</u>).

proteins in prawns and prawn products is cooking prawns to a minimum 70°C core temperature for at least 11 seconds.

The CA in the exporting country must certify on an official government health certificate that the cooked prawns:

- a) have been cooked in premises approved by and under the control of the CA and as a result of the cooking process, all the protein in the prawn meat has coagulated and no raw prawn meat remains
- b) are fit for human consumption.

# 16.2.6 Uncooked wild-caught prawns of Australian origin processed overseas in approved premises

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.

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 wild caught prawns of Australian origin, processed at a CA-approved establishment in accordance with the biosecurity integrity program agreed with the Australian Government Department of Agriculture, Water and the Environment
- 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 in Australia each batch of uncooked prawns will be subject to seals intact inspection and testing for WSSV and YHV1 at an approved screening laboratory at a rate determined by the department.

# 16.3 Review of processes

#### 16.3.1 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.

#### 16.3.2 Review of policy

The department can review the import policy at any time.

# 16.4 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.

# **Appendix 1**

# **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 draft 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. <u>Class 3.3 – Imported uncooked prawn product processing allows for processing of imported</u> 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. Compliance activities are outside the scope of this draft risk review. The department applies a range of regulatory tools to manage compliance, from 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 <u>Biosecurity Act 2015</u> 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 draft risk review.

#### Human consumption

Human consumption is the primary purpose for which prawns are imported. Of the hazards identified in this draft 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 draft 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 draft 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 draft 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 draft 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 draft 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 <u>Disposal of solids and liquid waste from</u> 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 draft 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 2**

# Legislation, policies and guidelines related to prawn biosecurity

# **Australian Capital Territory**

The Australian Capital Territory (ACT) did not provide information for this draft 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 <u>Health protocol for translocation of prawn post-larvae into NSW for</u> <u>stocking into NSW prawn farms for the 2019 season</u>. 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 draft risk review. The department notes that holders of licences issued in accordance with section 11 of the <u>Northern Territory</u> <u>Fisheries Act 1988</u> 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 <u>Health protocol for the movement of live prawns</u> 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 <u>Fisheries Act 1994</u>, 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 <u>Fisheries Act 1994</u> 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 restriction 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 the State. 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 the State 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 polychaete worms that originated in the WSD movement restriction area. Prawns from the WSD movement restriction 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 <u>Livestock</u> <u>Act 1997</u>). Livestock means animals kept or usually kept in a domestic or captive state including fish (by definition within the <u>Livestock Act 1997</u> 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 draft 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 <u>Fisheries Regulations 2019</u>. The regulations are made under the Victorian <u>Fisheries Act 1995</u>. 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 <u>Fisheries Regulations 2019</u> 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 <u>Fish Resources Management Act 1994</u> and the <u>Fish Resources Management Regulations</u> 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 <u>Biosecurity and Agriculture Management Act 2007</u> and the <u>Fish Resources Management Regulations 1995</u>. 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. 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 polychaete worms that are produced by aquaculture in Queensland or New South Wales, as well as crustaceans wild caught in Queensland from a restriction area.

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.

# Appendix 3

# Risk assessment values for unrestricted and restricted import of prawns

Table 18 shows the risk assessment values for unrestricted import and restricted (biosecurity measures applied) import for each hazard.

		Likelihood		of exposure likelihood of entry of					ıl likelil ablishm pread		Impa	ct							Likely conse	y equence	s	Partia	al annua	al risk	Annual risk	
Hazard	Biosecurity measure	od of entry	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	М	L	L	М	D	A	Е	D	С	A	В	М	L	L	N	N	N	N
	Unrestricted (chilled)	Н	L	L	М	L	L	М	М	L	L	М	D	A	Е	D	С	А	В	М	L	L	L	VL	L	L
CMNV	Unrestricted	Н	L	L	М	L	L	М	М	L	VL	М	Е	A	Е	D	В	Α	В	М	L	VL	L	VL	VL	L
	H&S removal	Н	VL	EL	М	VL	EL	М	М	L	VL	М	Е	А	Е	D	В	А	В	М	L	VL	VL	Ν	VL	VL
	Cooking	Н	N	N	VL	Ν	N	VL	М	L	VL	М	Е	А	Е	D	В	А	В	М	L	VL	Ν	Ν	Ν	N
	VAP	Н	N	N	EL	N	N	EL	М	L	VL	М	Е	А	Е	D	В	Α	В	М	L	VL	N	N	N	N
DIV1	Unrestricted	H	L	L	M	L	L	M	M	L	VL	M	E	B	E	D	C	E	B	M	L	VL	L	VL	VL	L
	H&S removal	H	VL	EL	M	VL	EL	M	M	L -	VL	M	E	B	E	D	C	E	В	М	L	VL	VL	N	VL	VL
	Cooking	H	N	N	VL	N	N	VL	M	L	VL	M	E	B	E	D	C	E	B	M	L	VL	N	N	N	N
FUD	VAP	H	N	N	EL	N	N	EL	M	L	VL	M	E	B	E	D	C	E	B	M	L	VL	N	N	N	N
EHP	Unrestricted	H	L	L	M	L	L	M	M	L	L	M	E	A	E	D	B	A	B	M	L	L	L	VL	L	L
	H&S removal	Н	VL	EL	М	VL	EL	М	М	L	L	М	E	А	Е	D	В	A	В	М	L	L	VL	N	L	L

#### Review of the biosecurity risks of imported prawns

		Likelihoc		al likeli posure	hood	likeli	al annua hood of xposure	entry	of est	al likeli ablishn pread		Impa	ct							Likely conse	/ quence	s	Partia	al annua	ıl risk	Annual risk
Hazard	Biosecurity measure	Likelihood of entry	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 + devein	М	VL	EL	М	VL	EL	L	М	L	L	М	Е	A	Е	D	В	А	В	М	L	L	VL	N	VL	VL
	Cooking	Н	N	Ν	VL	N	N	VL	М	L	L	М	Е	А	Е	D	В	А	В	М	L	L	Ν	Ν	N	N
	VAP	Н	N	Ν	EL	N	Ν	EL	М	L	L	М	Е	А	Е	D	В	A	В	М	L	L	Ν	Ν	N	N
IMNV	Unrestricted	Н	L	L	М	L	L	М	М	L	VL	М	С	Α	E	D	С	Α	В	М	L	VL	L	VL	VL	L
	H&S removal	Н	VL	EL	М	VL	EL	М	М	L	VL	М	С	А	Е	D	С	А	В	М	L	VL	VL	Ν	VL	VL
	Cooking	М	N	Ν	VL	N	N	VL	М	L	VL	М	С	А	Е	D	С	А	В	М	L	VL	N	N	N	N
	VAP	Н	N	Ν	EL	N	N	EL	М	L	VL	М	С	А	Е	D	С	А	В	М	L	VL	N	Ν	Ν	Ν
LSNV	Unrestricted	Н	L	L	М	L	L	М	М	L	VL	М	D	Α	E	D	В	Α	В	М	L	VL	L	VL	VL	L
	H&S removal	М	VL	EL	М	VL	EL	L	М	L	VL	М	D	А	Е	D	В	А	В	М	L	VL	VL	N	N	VL
	Cooking	Н	N	Ν	VL	N	N	VL	М	L	VL	М	D	A	Е	D	В	А	В	М	L	VL	N	N	N	N
	VAP	М	N	N	EL	N	N	EL	М	L	VL	М	D	A	Е	D	В	A	В	М	L	VL	N	N	N	N
TSV	Unrestricted	Н	L	L	М	L	L	М	М	L	VL	М	D	В	E	D	С	E	В	М	L	VL	L	VL	VL	L
	H&S removal	Н	VL	EL	М	VL	EL	М	М	L	VL	М	D	В	Е	D	С	Е	В	М	L	VL	VL	N	VL	VL
	Cooking	H	N	N	VL	N	N	VL	M	L	VL	M	D	B	E	D	C	E	В	М	L	VL	N	N	N	N
	VAP	Н	N	N	EL	N	N	EL	M	L	VL	M	D	B	E	D	C	E	B	M	L	VL	N	N	N	N
Vp AHPND	Unrestricted	VL	EL	EL	L	EL	EL	VL	H	L	M	H	F	A	E	E	C	A	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	A	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	A	B	H	M	H	N	N	VL	VL
	VAP	EL	N	N	EL	N	Ν	N	Н	L	М	Н	F	А	Е	Е	С	А	В	Н	М	Н	Ν	N	N	N

Appendix 3

#### Review of the biosecurity risks of imported prawns

		Likelihoo		al likelil bosure	hood	likeli	al annua hood of xposure	entry	of est	tial likelihood Impact establishment I spread									Likely conse	y equence	:S	Partial annual risk			Annual risk	
Hazard	Biosecurity measure	Likelihood of entry	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
WSSV	Unrestricted	Н	L	М	Н	L	М	Н	Н	М	М	Н	F	D	E	E	С	E	С	Н	Н	Н	М	Н	Н	Е
	H&S removal	Н	VL	EL	Н	VL	EL	Н	Н	М	М	Н	F	D	Е	Е	С	Е	С	Н	Н	Н	L	VL	Н	Н
	H&S removal + devein	Н	VL	EL	Н	VL	EL	Н	Н	М	М	Н	F	D	Е	Е	С	Е	С	Н	Н	Н	L	VL	Н	Н
	H&S removal + testing	L	VL	EL	Н	VL	EL	L	Н	М	М	Н	F	D	Е	Е	С	Е	С	Н	Н	Н	L	VL	М	М
	H&S removal + 2x testing	EL	VL	EL	Н	EL	N	EL	Н	М	М	Н	F	D	Е	Е	С	Е	С	Н	Н	Н	VL	N	VL	VL
	Cooking	VL	N	EL	VL	Ν	EL	EL	Н	М	М	Н	F	D	Е	Е	С	Е	С	Н	Н	Н	Ν	VL	VL	VL
	VAP	Н	Ν	Ν	EL	Ν	Ν	EL	Н	М	М	Н	F	D	Е	Е	С	Е	С	Н	Н	Н	Ν	Ν	VL	VL
YHV1	Unrestricted	Н	L	L	Н	L	L	Н	Н	L	L	Н	F	В	Е	Е	С	Е	С	Н	М	М	М	L	М	М
	H&S removal	Н	VL	EL	Н	VL	EL	Н	Н	L	L	Н	F	В	Е	Е	С	Е	С	Н	М	М	L	Ν	М	М
	H&S removal + devein	Н	VL	EL	Н	VL	EL	Н	Н	L	L	Н	F	В	Е	Е	С	Е	С	Н	М	М	L	N	М	М
	H&S removal + testing	L	VL	EL	Н	VL	EL	L	Н	L	L	Н	F	В	Е	Е	С	Е	С	Н	М	М	L	N	L	L
	H&S removal + 2x testing	EL	VL	EL	Н	EL	N	EL	Н	L	L	Н	F	В	Е	Е	С	Е	С	Н	М	М	VL	N	N	VL
	Cooking	Н	N	N	VL	Ν	Ν	VL	Н	L	L	Н	F	В	Е	Е	С	Е	С	Н	М	М	N	N	VL	VL
	VAP	Н	Ν	Ν	EL	Ν	Ν	EL	Н	L	L	Н	F	В	Е	Е	С	Е	С	Н	М	М	Ν	Ν	Ν	Ν

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:

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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. 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 5. All crustaceans: farmed, hatchery and wild crustacean exposure groups combined.

# **Appendix 4**

# **Batch definition**

For the purposes of testing prawns for pathogenic agents of biosecurity concern, a batch may be defined by one of the following (to be determined by the competent authority (CA)), but in any case, a batch cannot exceed 1 shipping container:

- product from a single line in a single processing run
- 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)
- 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 from the exporter, supplier or the CA 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 in order to determine the number of batches. This may result in additional testing and inspection costs.

# Glossary

Term or abbreviation	Definition
Approved arrangement (AA)	Approved arrangement (AA) is defined in the <i>Biosecurity Act 2015</i> 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 <i>Biosecurity Act 2015</i> 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.
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 <i>Biosecurity Act 2015</i> 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 <i>Biosecurity Act 2015</i> defines biosecurity measures as measures to manage any of the following: 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 <i>Biosecurity Act 2015</i> 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 OIE Code, or determined by the Department of Agriculture, Water and the Environment have been demonstrated.
CMNV	covert mortality nodavirus
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.

Term or abbreviation	Definition
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 <i>Biosecurity Act 2015</i> 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).
НАССР	Hazard Analysis Critical Control Point - a system that identifies, evaluates and controls hazards that are significant for food safety.
hazard	A biological agent in an aquatic animal or aquatic animal product with the potential to cause adverse consequences on 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 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
PCR	polymerase chain reaction
per os	Describes a treatment that is taken orally. From Latin 'by opening' or 'by way o 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
qRT-PCR	Quantitative (real-time) reverse-transcription-polymerase chain reaction

Term or abbreviation	Definition
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, Water and the Environment
TSV	Taura syndrome virus
unrestricted risk	Risk estimate without the application of biosecurity measures.
Vp AHPND	Vibrio parahaemolyticus strains containing Pir toxins
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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