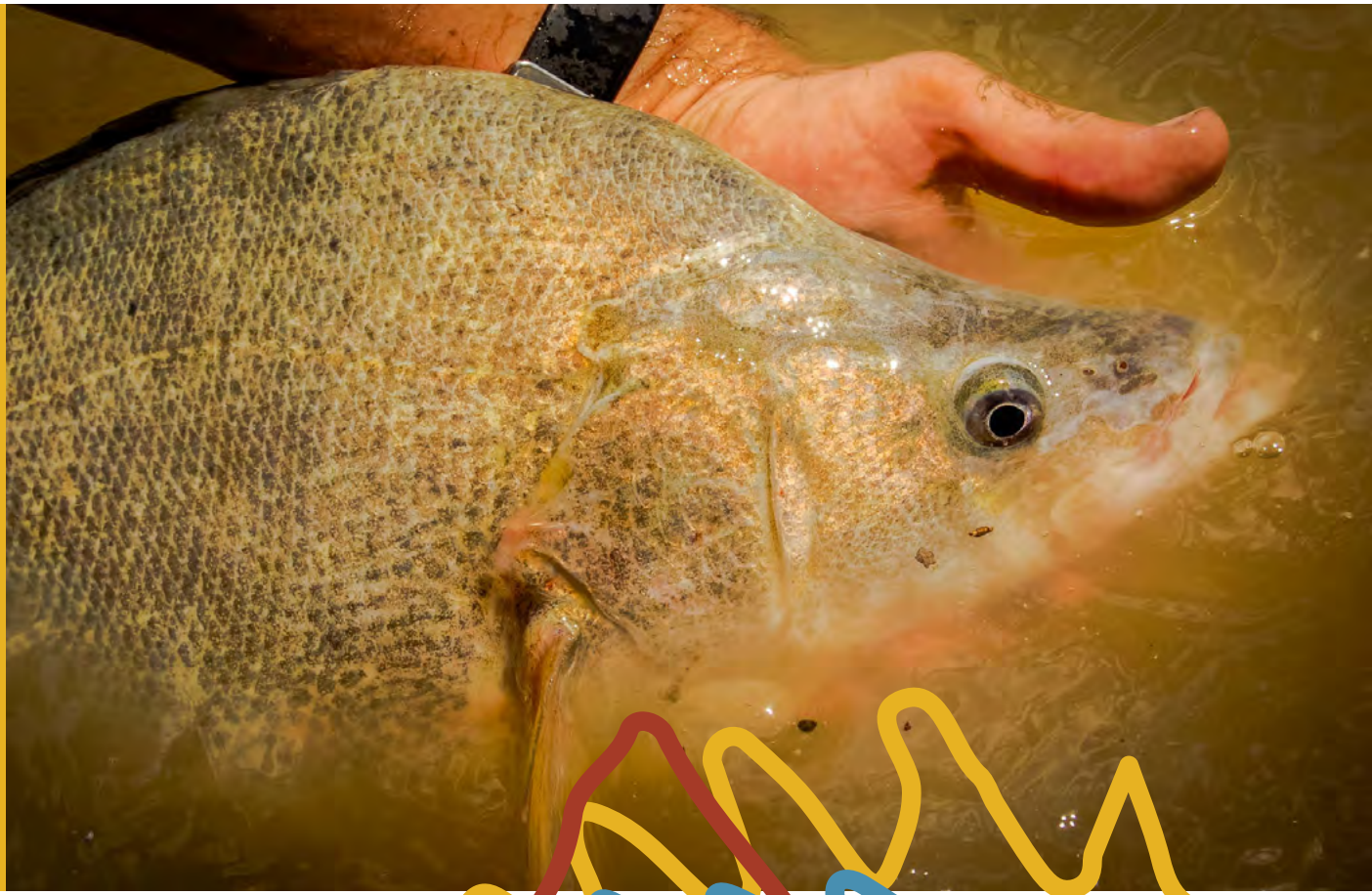
TECHNICAL PAPER 4

NATIONAL CARP CONTROL PLAN

Carp virus species specificity



This suite of documents contains those listed below.

NCCP TECHNICAL PAPERS

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2. Epidemiology and release strategies
3. Carp biocontrol and water quality
4. Carp virus species specificity
5. Potential socio-economic impacts of carp biocontrol
6. NCCP implementation
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Will carp virus biocontrol be effective?

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10. 2016-183: Cyprinid herpesvirus 3 and its relevance to humans
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Technical Paper 4: Carp virus species specificity

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1.0 About this paper

A virus called Cyprinid herpesvirus 3 (CyHV-3) has been proposed as a biological control agent for European Carp, or common carp (*Cyprinus carpio*, hereafter 'carp'), an invasive pest fish widespread in southeastern Australia. CyHV-3 belongs to the family Alloherpesviridae, which comprises viruses that infect fish and amphibians, and is the aetiological (causative) agent of one of 10 notifiable fish diseases listed by the World Organisation for Animal Health (OIE). The Australian Government has provided $10.211 million for development of a National Carp Control Plan (NCCP) assessing the viability of carp biocontrol using CyHV-3. Specificity to the target organism (in this case carp) is a fundamental criterion for a biological control agent. This paper describes and discusses key issues regarding CyHV-3 species specificity, drawing on information from the NCCP research program and the broader scientific literature.

2.0 Viral infection, host range, and host switching: overview and definitions

2.1 Introducing viral infection

Viruses are obligate intracellular pathogens. To reproduce, they must enter the cells of a host organism and use the molecular machinery (organelles) contained therein to produce more virus copies in a process called replication (Butel, 2013). In other words, viruses can only reproduce by hijacking host cells and forcing them to produce more virus particles. As replication proceeds, the infected cell either bursts open (lyses), or pockets of virus particles bud off from the infected cell, enabling infection of surrounding cells (Grinde, 2013). Virions (complete, protein-coated virus

particles) can usually remain viable and infectious outside a host cell for time periods that vary between viral species (Pirtle and Beran, 1991; Weber and Stilianakis, 2008). However, these virions are not replicating, but merely persisting, and will become non-viable if they do not gain access to appropriate host cells (Pirtle and Beran, 1991). Disease is not an automatic consequence of viral infection; even apparently healthy hosts may carry diverse microbial communities (Geoghegan and Holmes, 2018).

2.2 Viral host range

The species a virus can infect constitute its 'host range'. Some viruses have a host range that is restricted to a single species, or just a few species (Bandín and Dopazo, 2011). Host range restriction occurs because different hosts present distinct molecular and immunological contexts that an invading virus must negotiate to infect host cells (Parrish et al., 2008; Bandín and Dopazo, 2011; Lee et al., 2016). For example, the process by which a virus enters a cell involves complex biochemical interactions between the virus and molecules called cell surface receptors on the cell membrane; if an invading virus cannot bind to a potential host's cell surface receptors, it cannot infect the cell (Parrish et al., 2008; Longdon et al., 2014). Similarly, host immune systems surveil for, and neutralise, invading pathogens, so a virus must possess the ability to evade or defeat these defences if it is to establish an infection (Parrish et al., 2008; Sharp and Hahn, 2011; Lee et al., 2016). The viral adaptations necessary to optimise infection, replication, immune evasion or suppression, and onward transmission in one host species are generally not broadly applicable across other potential host species, producing restricted viral host ranges (Parrish et al., 2008; Bandín and Dopazo, 2011; Sharp and Hahn, 2011).

Some viruses have naturally broad host ranges (i.e. can infect many species). For example, viral haemorrhagic septicaemia virus (VHSV) infects a diverse range of marine and freshwater fish species, while Bohle iridovirus (BIV), a ranavirus, infects both fish and frog species (Hedrick et al., 2003; Bandín and Dopazo, 2011).

Translocating viruses with broad host ranges, whether intentionally as part of a biocontrol program, or unintentionally in the course of trade or travel, raises concerns because these movements could bring a novel virus into contact with prospective host species that are part of its host range, but that have previously not been infected simply because host and virus have never made contact (Hedrick et al., 2003; Parrish et al., 2008; Di Giallonardo and Holmes, 2015). A broad host range is generally an undesirable trait in a potential biocontrol virus, because the virus's inherent capacity to infect diverse hosts usually increases the likelihood of infection, and possibly disease, in non-target species (NTS) (DiGiallonardo and Holmes, 2015).

2.3 Viral host switching

A virus and its established host specie(s) have a 'host-pathogen relationship'. In many instances, particularly for viruses with double-stranded DNA (dsDNA) genomes, these relationships have evolved over thousands, or millions, of years, and represent an equilibrium in which the virus does not significantly harm the host (Geoghegan and Holmes, 2018). Under some circumstances, however, viral genomes change in ways that enable a shift from the established host or hosts into a new species that was not previously part of the virus's host range. These events are interchangeably termed 'host jumps', 'species jumps' or 'host switches'. In a viral host switch, the established host is called the 'donor', and the new host is called the 'recipient' (Parrish et al., 2008). Viruses may jump directly from the donor to the recipient host, or may pass through an intermediate host (Parrish et al., 2008).

Host switching demands that a virus acquires the adaptations necessary to infect a new host species (Holmes, 2013a,b). These adaptations are acquired through one or more mechanisms of viral evolution, such as mutation, recombination, or reassortment (Box 1, at end of paper). The changes to the viral genome involved in these adaptations distinguish a host switch from the situation in which a

virus infects a species that was already part of its host range, but, due to lack of opportunity rather than the virus's inability to infect the host, had hitherto not been infected.

Successfully switching hosts is a challenging evolutionary feat for a virus, requiring

- the acquisition of adaptations that enable infection of a new host, yet do not negatively affect viral fitness,
- subsequent selection favouring the new viral variants, and
- ecological and/or social circumstances that favour repeated contact, of a type enabling viral transmission, between the donor and recipient hosts (Holmes, 2013a,b; Longdon et al., 2014).

Furthermore, the initial infection of a new recipient host is only the first stage in a successful host switch; acquiring the capacity for successful onward transmission is usually more difficult than the initial switch (Wain-Hobson, 1998; Holmes and Drummond, 2007; Lee et al., 2016; Geoghegan and Holmes, 2018). For these reasons, many host-switching events never proceed past the 'spillover' stage (Wain-Hobson, 1998; Parrish et al., 2008). In spillover infections, the donor host species forms the reservoir within which the virus circulates, occasionally jumping into the recipient species, but failing to onwardly transmit in the new host ('dead-end' infections), or only establishing short chains of local transmission that quickly fade out. Attaining self-sustaining transmission in the recipient host (i.e. transmission that does not require the ongoing presence of a donor host reservoir) typically requires numerous spillover infections, one or more of which 'takes' when a well-adapted viral variant spills over into the recipient population under ecological conditions suitable for onward transmission (Holmes and Drummond, 2007; Parrish et al., 2008). The virus may then establish epidemic or endemic transmission in the recipient host. Epidemic transmission typically covers broad geographic areas, and involves numerous approximately simultaneous infections. Endemic diseases constantly circulate in the host population in a relatively stable manner.

Despite the evolutionary challenges that host-switching poses to viruses, numerous viral host switches have occurred through evolutionary time, and will continue to occur, probably with increasing frequency as global change mediates ecological disturbance and creates new conjunctions of potential donor and recipient hosts (Parrish et al., 2008; Parvez and Parveen, 2017). Most of these events will go unreported (Parvez and Parveen, 2017). Indeed, host-switching is not simply a by-product of viral evolution, but an important driver of it, and phylogenetic analyses examining the respective evolutionary 'family trees' of viruses and their hosts through long time periods reveal that host-switching is almost ubiquitous, although the rates at which it occurs differ among viral lineages (Bandín and Dopazo, 2011; Geoghegan et al., 2017).

3.0 OIE position on CyHV-3 species-specificity

The World Organisation for Animal Health (OIE) in its *Manual of Diagnostic Tests for Aquatic Animals* (OIE (2022), hereafter 'the Manual') lists susceptible host species as all varieties and subspecies of common carp, and hybrids thereof. Species for which there is insufficient evidence to fulfil the criteria for listing as susceptible are Goldfish (*Carassius auratus*), Grass Carp (*Ctenopharyngodon idella*), and Crucian Carp (*Carassius carassius*). The Manual further notes positive PCR tests without evidence of active infection in the following taxa:

- three fish species (including a hybrid) from the family Acipenseridae (sturgeons),
- four fish species from the family Cyprinidae (carps and carp-like fishes),
- one fish species from the family Nemacheilidae (loaches),
- two fish species from the family Percidae (perches),
- a crustacean (Scud, *Gammarus pulex*), and

- a mollusc (Swan Mussel (*Anodonta cygnea*).

The Manual's chapter on Infection with Koi Herpesvirus (Chapter 2.3.6) can be viewed at https://www.woah.org/fileadmin/Home/eng/Health_standards/aahm/current/2.3.06_KHV.pdf. Please note that the Manual is periodically updated as new information becomes available. The version consulted during preparation of this paper was adopted in May 2022.

4.0. Species-specificity research in the NCCP

There are four projects either commissioned by, or directly informing, the NCCP that assist in assessing the risk that CyHV-3 will infect non-carp species. Three projects focus on risk of CyHV-3 infection in non-human animals other than common carp, and one assesses the potential for CyHV-3 infection in human beings.

4.1 Research investigating the potential for human infection by CyHV-3

As part of the NCCP research program, Roper and Ford (2018) systematically searched medical databases for evidence of human infection by CyHV-3, and did not find any reported cases. The review concluded that human infection by CyHV-3 is extremely unlikely. Average human body temperature (~36.1–37.2 °C) lies outside the virus's permissive temperature range, which is variously cited as 18–28 °C (Michel et al., 2010; Gotesman et al., 2013; Rakus et al., 2013) and 16–26 °C (Hanson et al., 2016; see discussion in Becker et al. (2018)). This disjunction between the virus's permissive range and human body temperature precludes infection even before the physiological and immunological differences between humans and fish that would present barriers to host switching are considered (see Holmes (2013a,b) and Wain-Hobson (1998) for relevant discussions). In the following discussions, the acronym 'NTS' (non-target species) refers to animal species other than common carp.

4.1.1 Implications

The risk of direct human infection by CyHV-3 is so negligible as to be considered non-existent. No further research in this area is recommended.

4.2 Research investigating the potential for CyHV-3 to infect animals other than carp

4.2.1 CSIRO viral challenge trials

Over approximately eight years to 2016, CSIRO researchers at the Australian Animal Health Laboratories (AAHL), supported by funding from the Invasive Animals Cooperative Research Centre (IACRC), tested the susceptibility of 22 species to infection by an Indonesian strain of CyHV-3 (McColl et al., 2016). Trials such as these, which test susceptibility of selected species to a specific viral strain under specific experimental conditions, aim to enable inferences about whether the test species are part of the viral strain's host range. Such trials do not test whether the virus could, at some future stage, evolve in ways that enable infection of a new species (i.e. host switching).

Species tested in the CSIRO trials comprised 13 Australian native fishes, introduced Rainbow Trout, a lamprey, a crustacean (freshwater yabbies, *Cherax destructor*), two frog species, two native reptiles (a freshwater turtle and a water dragon), chickens (a representative bird), and mice (a representative mammal) (McColl et al., 2016). Species selected for testing included representatives of most taxonomic orders that would be exposed to CyHV-3 if it were released in Australian ecosystems (McColl et al., 2016). The rationale for species selection was discussed with, and approved by, the Australian Pesticides and Veterinary Medicines Authority. Wherever possible, both adults and

juveniles of each species were tested, with exposure occurring through injection of virus into the body cavity, and/or by immersing test animals in tanks containing high virus concentrations ('bath exposure') (McColl et al., 2016). Some delicate species, such as Australian Smelt (*Retropinna semoni*, a small native fish), were unable to survive the physical stress associated with direct injection, and therefore only underwent bath exposure.

Diagnostic protocols included histopathological examination of NTS tissues, attempts to isolate CyHV-3 in cell cultures, standard Polymerase Chain Reaction (PCR) assays that detect viral DNA, and a Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay designed to detect mRNA from the CyHV-3 terminase gene (Yuasa et al., 2012; McColl et al., 2016). Detecting CyHV-3 mRNAs in NTS provides strong evidence of a replicative infection, because expression of viral genes as functional mRNAs early in infection is essential for synthesis of viral proteins (Rampersad and Tennant, 2018). The essential role of mRNA in viral replication means that detection of viral mRNA strongly indicates that the virus has invaded host cells and is replicating (i.e. has infected the host) (Yuasa et al., 2012). In contrast, detecting a virus's genomic DNA in a potential host's tissues proves that the virus is present, not necessarily that it is replicating.

McColl et al. (2016) found no evidence of replicating CyHV-3 in any of the tested NTS. Nonetheless, as with most research, some questions remained. These questions are explored in detail by Pyecroft and Jones (2020), but two of the most important are briefly summarised here. First, CyHV-3 genomic DNA was detected by PCR in some NTS. Subsequent RT-PCR assays did not detect CyHV-3 terminase gene mRNA in any of these individuals. McColl et al. (2016) interpreted these results as indicating that CyHV-3, while physically present, had not infected the NTS.

Second, experimental groups of Rainbow Trout (*Oncorhynchus mykiss*), Sea Mullet (*Mugil cephalus*), Silver Perch (*Bidyanus bidyanus*), and Peron's tree frog (*Litoria peronii*) tadpoles exposed to the virus experienced higher mortality rates than their corresponding control groups (i.e. those that underwent all experimental procedures other than virus exposure) (McColl et al., 2016). Of these species, Rainbow Trout, Sea Mullet, and Silver Perch were exposed to CyHV-3 via both bath and injection, while Peron's tree frog tadpoles were exposed only via bath (McColl et al., 2016). The mortalities observed in treatment groups for these species could indicate an effect of the virus (McColl et al., 2016). However, RT-PCR did not detect CyHV-3 mRNA in any of these fishes, indicating that they were not infected by the virus, but the mortalities remain unexplained.

Throughout the study, no NTS exhibited pathological signs (neither gross nor histological) consistent with CyHV-3 infection in carp (McColl et al., 2016). Similarly, attempts to isolate CyHV-3 from Silver Perch, Golden Perch, and Murray Cod at various periods post-exposure were unsuccessful, suggesting lack of infection in these species (McColl et al., 2016). CyHV-3 is, however, difficult to propagate in cell cultures, so this result does not reliably indicate absence of infection (Pyecroft and Jones, 2020).

The viral challenge trials of McColl et al. (2016) provided evidence indicating that CyHV-3 only infects common carp. Nonetheless, because species-specificity is so fundamental to decision-making on carp biocontrol, and in response to advice from the NCCP Science Advisory Group and stakeholder questions about the points described above, a review of best-practice in viral challenge trials for CyHV-3 was commissioned as part of the NCCP research program.

4.2.2 Review of best practice in viral challenge for CyHV-3

The review (Pyecroft and Jones, 2020), critically appraised NTS susceptibility research for CyHV-3, and aimed to develop best-practice recommendations for any future testing. The review covered the following topics:

(i) Identifying suitable diagnostic approaches/techniques for determining the resistance status of NTS to CyHV-3 infection.
(ii) Identifying suitable approaches for addressing unexplained mortalities and false positives when testing NTS resistance to CyHV-3 infection. In viral challenge trials, a 'false positive' result refers to apparent virus detection in NTS by molecular assays such as PCR, but with subsequent investigation failing to find any evidence of viral presence and/or infection.
(iii) Determining whether stressors should be deliberately applied when assessing NTS resistance to CyHV-3 infection.
(iv) Assessing the NTS life history stages (i.e. larval, juvenile, adult) that should be tested for susceptibility to CyHV-3 infection.
(v) Assessing the potential for NTS, other than those previously investigated, to become infected by CyHV-3.
(vi) Following from objective (v), assessing whether future NTS susceptibility testing should include a wider range of NTS than those already tested.

Pyecroft and Jones (2020) defined viral infection as *"…the presence of a multiplying or otherwise developing or latent viral agent in a host. It may cause no clinical signs (subclinical infection) or it may cause signs that are clinically apparent*". This emphasis on viral replication (even if followed by a non-replicative latent infection), as the essential feature of viral infection is consistent with the fundamental aspects of virus biology as described in section 2.1 of this paper.

In addition to technical critiques and recommendations regarding laboratory techniques for identifying CyHV-3 infection (molecular assays for detecting viral DNA and RNA, isolation in cell culture, serology), the review concluded that CyHV-3 could potentially infect some non-target fish species (both Australian native species and those from the northern hemisphere), though without causing clinical signs of disease (Pyecroft and Jones 2020). The review's authors based this conclusion on (i) detection of CyHV-3 DNA in NTS (while acknowledging that this does not in isolation constitute evidence of infection), and (ii) the apparent ability of NTS exposed to CyHV-3 to transmit the virus to naïve carp through co-habitation (again, generally without clinical disease signs in the carp). The review further concluded a range of life-history stages from representatives of every freshwater and inshore taxonomic family that could be exposed to CyHV-3 if the virus were released into the Australian environment should ideally be tested for susceptibility to infection (but noting that logistical constraints could preclude testing so many species).

The review's conclusions stimulated extensive discussion among the NCCP Science Advisory Group (SAG). The SAG unanimously accepted the review's conclusion that further non-target species susceptibility testing was warranted, but noted that the review had not met the objective of determining best practice in non-target species susceptibility testing (as defined through the OIE) through a practical set of targeted recommendations, but had instead provided broad advice for testing of non-target species resistance. Of particular concern was the broad and impractical recommendation that representatives of all taxonomic families that could potentially be exposed to the virus be tested. Implementing this recommendation would necessitate an essentially endless, and extremely costly, research program testing taxa as diverse as carcharhinid sharks, muraenesocid eels, and large, fast-moving fish from families such as Carangidae and Elopidae. No Australian research facilities accredited to handle CyHV-3 have the capacity to house these animals, and, more importantly, the costs of such an extensive testing program would be prohibitive. On the basis that such broad-ranging recommendations did not substantially develop or advance a useful pathway for future NTS research, the SAG did not formally accept the review, but did recommend additional NTS trials.

4.2.3 Additional non-target species susceptibility testing

Consistent with the recommendation by Pyecroft and Jones (2020) that additional non-target species susceptibility testing was required, a project was initiated in late 2019 to re-test Murray Cod, Silver Perch, and Rainbow Trout for susceptibility to CyHV-3 infection at CSIRO's Australian Centre for Disease Prevention (ACDP) (Moody et al., 2022). This second round of testing was delayed considerably by the COVID-19 pandemic, during which time ACDP facilities necessarily prioritised COVID-19 research. Once the NTS trials began, Rainbow Trout began to experience major mortalities on arrival at ACDP, well before any exposure to CyHV-3. Subsequent investigations identified that municipal water treatment processes had changed to incorporate the use of chloramine, without the investigators' knowledge, and that the consequent presence of chlorine in the facility's aquaria had killed the trout. By the time the cause of the mass rainbow trout mortalities was discovered, the CSIRO Animal Ethics committee had directed that the experiment should proceed without this species. Rainbow Trout were consequently not included in the testing, and knowledge of their susceptibility or otherwise to infection by CyHV-3 was therefore not advanced by the project.

Testing proceeded for Murray Cod and Silver Perch, and no evidence of infection was detected in these two species (Moody et al., 2022). As the original NCCP SAG had completed its functions and ceased meeting in late 2019, this project report (completed mid-2022) was considered by a smaller advisory group, referred to as the 'NCCP Special SAG'. The Special SAG included members with the subject-matter expertise necessary to assess the remaining projects, as well as those with broad scientific interests across NCCP research and its implications. The Special SAG did not accept this project report. The Special SAG's reasons for not accepting the work included the omission of Rainbow Trout from the testing, which meant the project could not fully meet its objectives, mortalities in both test (i.e. exposed to the virus) and control (not exposed to the virus) fish that, while apparently not due to infection by the virus, could not be definitively attributed to other causes, and generally insufficient data to support a determination of susceptibility or resistance in test fish.

Additionally, community concern regarding infection of non-target species by the carp virus is relatively common, with 57% of 4680 survey respondents concerned that the virus might be transmissible to fish or animals other than carp (Schirmer et al. 2019). International research has also raised questions regarding the susceptibility of Rainbow Trout to CyHV-3 infection (Bergmann et al. 2020). Given (i) the non-acceptance of the second round on NTS susceptibility testing by the Special SAG, and (ii) the prevalence of community concerns regarding the virus's specificity to carp, the NCCP recommends additional NTS susceptibility trials before a final decision on virus release is made.

4.2.4 Implications

Species-specificity research under the NCCP has focussed on trials that test whether selected species form a hitherto undetected component of CyHV-3's host range. These trials test the susceptibility of selected species to infection with a specific viral strain, under a single set of laboratory conditions. Regardless of how carefully designed and meticulously conducted such trials may be, they consequently do not provide definitive evidence of a tested species' resistance to viral infection under all conditions. These caveats do not diminish the value of well-planned viral challenge trials; results from this research are essential precursors to any biological control program, and provide useful insights into the likely species-specificity of a prospective biocontrol agent. Further trials of this nature are recommended before decision-making on virus release. These trials should include Rainbow Trout, but a limited range of other species could also be identified for inclusion through consultation with stakeholders and scientific experts.

Challenge trials do not provide insights into a virus's future evolutionary trajectory, including the possibility that evolutionary changes to the viral genome over time could enable host-switching to infect a new species. Indeed, predicting future host-switching events is so difficult that some researchers who study virus evolution caution against attempting it; rather, they suggest, effort may

best be allocated to surveillance efforts aimed at early detection of, and response to, host-switching events (see discussions in Holmes (2013b), Geoghegan and Holmes (2017), and van der Hoek et al. (2018)). Given this complexity, there can be no absolute guarantees that CyHV-3, or indeed any other virus, will never switch hosts to infect a new species.

Nonetheless, there is considerable evidence to suggest that CyHV-3 presents a very low host-switching risk. CyHV-3 is a dsDNA virus, and at 295,146 base pairs, its genome is the largest in the family Alloherpesviridae (Davison et al., 2013). In general, viruses with large dsDNA genomes tend to adopt an evolutionary strategy based on co-divergence and co-existence with their host, rather than frequent switching between hosts (Geoghegan et al., 2017). Through evolutionary time (i.e. tens of thousands to millions of years), these periods of co-divergence are usually punctuated by host-switches, but these are much less frequent than for small, single-stranded RNA viruses that tend to switch hosts frequently (Geoghegan et al., 2017).

The contention that alloherpesviruses are likely to co-diverge with their hosts for extended time periods is supported by phylogenetic analyses, which reconstruct host and virus 'family trees' through evolutionary time. Phylogenetic analysis of the alloherpesviruses revealed evidence of host switching at deeper (i.e. older) nodes of the phylogenetic tree (Waltzek et al., 2009). In particular, alloherpesviruses appear to have switched between sturgeons (family Acipenseridae) and catfishes (family Ictaluridae), and between cyprinid fishes (carp, Goldfish etc) and eels (family Anguillidae) in the ancient past (Waltzek et al., 2009; Bandín and Dopazo, 2011). There is, however, little evidence of more recent host-switching, with cyprinid, ictalurid (catfish), salmonid (trout and salmon), and ranid (frog) herpesviruses segregating with the corresponding branches of their respective host phylogenies (Waltzek et al., 2009).

Practical experience with CyHV-3 internationally is also indicative of species specificity. Since outbreaks began in the mid-1990s, disease has only been reported in European Carp, despite the presence in northern-hemisphere aquatic ecosystems of numerous fish species closely related to carp (Thresher et al., 2018). The absence of observed disease in species other than carp does not preclude the possibility of unnoticed or unreported spillover events (see discussions in Parvez and Parveen (2017) and Geoghegan and Holmes (2018)). Nonetheless, the absence of reported disease in species other than carp over the last ~24 years is consistent with specificity to carp.

Nor is the initial emergence of CyHV-3 in carp aquaculture necessarily indicative of a host-switch. The mechanisms underlying CyHV-3's emergence are unclear, but there is some indication that CyHV-3 may have circulated among wild carp populations before appearing in aquaculture (Uchii et al., 2014). This contention is supported by close alignment between the respective life cycles of CyHV-3 and common carp (Uchii et al., 2014). Permissive temperatures for CyHV-3 replication, and consequently for infection, align with seasons when carp are aggregating to spawn, thereby creating ideal conditions for transmission (Uchii et al., 2014). The apparently close adaptation of CyHV-3 to its host's life cycle may indicate a relationship between carp and the virus through evolutionary time, although this is not proven (Uchii et al., 2014).

In summary, NCCP research on CyHV-3 species-specificity has focussed primarily on trials that aim to determine whether tested species are part of the virus's host range. These trials are essential precursors to release of any biocontrol agent. Nonetheless, challenge trials cannot provide information about a virus's longer-term evolutionary trajectory, including the potential for evolutionary changes that could lead to host-switching. Consequently, host-switching can never be completely discounted as a possibility for any virus. CyHV-3 does, however, possess a range of traits that suggest host-switching presents a low risk. Thus, decision-making on CyHV-3 release will unavoidably involve value-judgements in which a likely small, but ultimately unquantifiable, host-

switching risk is weighed against the potential environmental and economic benefits that could accrue from carp control.

5.0 Conclusions

Species-specificity is a fundamental prerequisite for most biocontrol agents. In the context of a viral biocontrol agent like CyHV-3, species-specificity can be broken down into two broad questions. Question one relates to the virus's host range—the diversity of species the viral strain or strains proposed for use as a biocontrol agent is capable of infecting *in its current form*. Questions about host range can be addressed through challenge trials, in which selected NTS are exposed to the virus in the laboratory to see if infection occurs. CyHV-3 challenge trials conducted by CSIRO did not find any evidence of CyHV-3 infection in 22 tested species, spanning fishes, frogs, crustaceans, reptiles, lampreys, mammals (mice), and birds (chickens). Nonetheless, further testing is recommended to ensure this vital question is thoroughly addressed.

Question two asks whether the virus's genome could evolve following release in a way that enables infection of new host species (host-switching). Predicting viral evolution is extremely complex, and host-switching events can never be completely discounted for any virus. However, both international experience with CyHV-3 and the virus's basic biological traits indicate that imminent host-switching by CyHV-3 is unlikely. Specifically, large, dsDNA viruses like CyHV-3 tend to adopt an evolutionary strategy based on long periods of co-divergence with their host species (Geoghegan et al., 2017).

6.0 References

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Box 1 text Definition of terms for virus evolution

Mutation: Viral reproduction, called replication, involves using the cellular machinery (organelles) of an infected host to produce new virus copies. Sometimes, mistakes occur in the biochemical process of copying viral nucleic acids (RNA and DNA). These mistakes are mutations. Most mutations simply result in ineffective viral particles that die immediately, but, by random chance, a mutation occasionally appears that enables infection of a new host.

Recombination: A mechanism of viral evolution that occurs when two different viruses infecting a host cell at the same time exchange genetic material, giving rise to a new viral variant. The new variant is referred to as a 'recombinant' virus. Recombination rarely results in host switching, but can occasionally do so.

Reassortment: A mechanism of viral evolution conceptually similar to recombination, but involving only viruses that have segmented genomes (e.g. influenza viruses).

Box 2 text Defining latent and chronic productive infection

Latency and subclinical infection are virologically distinct but, in the particular context of carp biocontrol, have similar epidemiological implications. In virological terms, 'latency' refers to a strategy used by some viruses, including herpesviruses, to evade from their host's immune system when conditions are unsuitable for active viral replication (Reed et al., 2014; Serquiña and Ziegelbauer, 2016). The exact mechanism viruses use to establish and maintain latency within an infected host varies between viral families (Serquiña and Ziegelbauer, 2016). In herpesvirus latency, the virus forms a circular genetic element called an episome that hides inside host cells, thereby avoiding discovery and attack by the host immune system. Episomes multiply along with the host cells during normal host cell division, but do not replicate by 'hijacking' the host cells. When conditions again become suitable for the virus to hijack host cells (for example, the host immune system becomes weakened), the virus emerges from latency and active replication recommences (Reed et al., 2014; Serquiña and Ziegelbauer, 2016). This active replication phase is called the 'lytic' cycle, because this is when the replicating virus particles either 'lyse' (burst open), or bud off from infected cells (Grinde, 2013). Thus, herpesviruses have a latent phase, when the virus is hiding in host cells, and a lytic phase, when the virus is actively replicating (Reed et al., 2014; Boutier et al., 2015; Reichert et al., 2019). Infectious virus is not produced during latent herpesvirus infection, a generalisation that, based on laboratory trials, appears to extend to CyHV-3 (Sunarto et al., 2014; Hanson et al., 2016).

In contrast to latency, subclinical infection does not involve sequestration of the virus in an episome. Rather, the virus continues to replicate in host cells, but does so at low levels that do not cause clinical signs of disease, and does not 'aggravate' the host immune system into an aggressive response (Grinde, 2013; Sunarto et al., 2014). Thus, subclinical infections are a 'toned down' lytic infection (Sunarto et al., 2014). Subclinical infections are also termed 'chronic productive' infections, because they are persistent through time (chronic) and involve viral replication (so they 'produce' new virus particles).

CyHV-3 infection can undoubtedly follow a trajectory that is highly indicative of latent and/or subclincal infection. Diseased carp recover when temperatures move out of the permissive range, yet continue to test positive for virus presence, and may subsequently re-develop lytic (and sometimes fatal) infections, with onward transmission to susceptible carp, when temperatures re-enter the permissive range (Sunarto et al., 2014; Boutier et al., 2015). Whether these characteristics indicate true latency, or persistent subclinical infection has not been completely resolved (Michel et al., 2010; Sunarto, 2014). A gene important in controlling latency in mammalian herpesviruses has not been found in fish herpesviruses, potentially indicating chronic productive infection rather than true latency (Sunarto et al., 2014). Conversely, there is evidence that carp white blood cells could be the location where latent virus 'hides' from the host immune system (Michel et al., 2010; Eide et al., 2011; Xu et al., 2013; Reed et al., 2014). Regardless of whether the carp virus exhibits true latency or chronic productive infection, carp in this phase of infection do not appear to produce infectious virus (Sunarto et al., 2014).

NATIONAL CARP CONTROL PLAN

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