National Carp Control Plan Issues Paper 3: Cyprinid herpesvirus 3 (CyHV-3) species specificity

Introductory section common to all papers

The National Carp Control Plan (NCCP) is being developed to examine and make recommendations about the feasibility of using a virus to assist in controlling common carp in Australia. The plan is to be developed by December 2019. Although focussed primarily on viral biocontrol, the NCCP will also make recommendations about the investigation and potential future use of other carp control methods.

This issues paper is one of seven prepared to summarise topics central to the NCCP's development, provide updates on emerging research results, and, where relevant, situate NCCP research within the broader context of scientific literature. Some papers within the series are intended primarily to provide background information or updates, whereas others seek stakeholder input to help shape development of the National Carp Control Plan document. An NCCP engagement report will be completed and published summarising stakeholder input.

The papers draw on results from the NCCP research program, the broader scientific literature, and stakeholder knowledge. Paper topics are:

(i) Why and how did the National Carp Control Plan originate?
(ii) What is science telling us about the potential use of the carp virus as a biological control agent?
(iii) Species-specificity of the carp virus
(iv) Managing water quality and associated issues
(v) Clean-up options
(vi) Social and economic impact assessment
(vii) Genetic biocontrol and common carp

Each of the papers can be read in sequence or singly. Many of the important questions and challenges associated with carp control are multidisciplinary and multifaceted, so cross-referencing between papers is used to direct readers towards more detailed discussions of a particular topic, or to Frequently Asked Questions (FAQs) on the NCCP website (http://www.carp.gov.au/FAQ), when necessary.

Common or European carp (*Cyprinus carpio*, referred to simply as 'carp' in these papers) are an introduced pest fish common throughout a large area of Australia. When carp are abundant, they can damage aquatic ecosystems in several ways, generating environmental, economic and social costs. Carp control initiatives in Australia are therefore based on the general premise that reducing carp numbers below the densities at which they cause environmental damage could result in improved environmental, social, and economic outcomes. While there is evidence for environmental improvements following carp control, these may not eventuate in all ecosystems, follow uniform transition pathways from the 'pre-control' to 'carp controlled' states, or be achieved without activities to address other, non-carp impacts.

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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 common or European carp (*Cyprinus carpio*), 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 12 notifiable fish diseases listed by the World Organisation for Animal Health (OIE). The Australian Government has provided $10.211 million over three years 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 common 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 persist through time as coherent taxonomic units, they must enter the cells of another species and use the molecular machinery (organelles) contained therein to produce more virus copies in a process is 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 host immune 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 the 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; DiGiallonardo 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 organisms (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). 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

(i) the acquisition of adaptations that enable infection of a new host, yet do not negatively affect viral fitness,
(ii) subsequent selection favouring the new viral variants, and
(iii) 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 propitious 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 and Holmes, 2017a).

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 (2018), hereafter 'the Manual') notes that naturally-occurring CyHV-3 infections have only been recorded in common carp (including ornamental variants) and hybrids thereof. The Manual further notes that a range of non-carp fish and aquatic invertebrates can vector CyHV-3, but does not make a determination upon whether this vectoring involves replication in the vector species or is purely mechanical (i.e. vector species carry CyHV-3 virions on their bodies in ways that enable infection of carp, but are not themselves infected). The manual also notes some evidence indicating potential CyHV-3 infections in goldfish (*Carassius auratus*). The susceptibility status of goldfish has been debated elsewhere (Yuasa et al., 2013).

4.0. Species-specificity research in the NCCP

There are three projects either commissioned by, or directly informing, the NCCP that assist in assessing the risk that CyHV-3 will infect non-carp species. Two 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

While few biological events are completely impossible, 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

Ove approximately eight years to 2016, CSIRO researchers at the Australian Animal Health Laboratories (AAHL) 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), 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 which 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 mRNA is an essential intermediate molecule of DNA replication. During replication, viral DNA cannot be copied directly, but must first be transcribed into mRNA. The essential role of mRNA as an intermediary 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). 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 (2019), 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, potentially indicating 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, 2019).

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, 2019), critically appraised NTS susceptibility research for CyHV-3, and developed best-practice recommendations for any future testing. Topics covered by the review are

(i) Appropriate techniques for diagnosing CyHV-3 infection.
(ii) Development of best-practice approaches for investigating undiagnosed deaths among NTS exposed to CyHV-3.
(iii) Development of best-practice approaches for addressing false positives in NTS exposed to CyHV-3. 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.
(iv) Determining whether stressors should be deliberately applied when assessing susceptibility of NTS to CyHV-3 infection.
(v) Assessing the NTS life history stages (i.e. larval, juvenile, adult) that should be tested for susceptibility to CyHV-3 infection.
(vi) Determining how many species should be tested for susceptibility to infection with the carp virus, and what criteria should be used for their selection.

A draft of this review has been completed and is currently progressing through peer review. Broadly, the review has recommended some additional work to improve confidence in CyHV-3's species specificity.

4.2.3 Implications

Species-specificity research under the NCCP has focussed on trials that test whether selected Australian native 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.

Additionally, 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 (2017b), 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 and Holmes, 2017a). 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 and Holmes, 2017a).

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 common 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 carp for extended periods as a relatively harmless virus before conditions in intensive aquaculture caused it to increase in virulence (i.e. severity or harmfulness) without crossing a species barrier. This contention is supported by close alignment between the respective life cycles of CyHV-3 and common carp (Uchii et al., 2004). 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., 2004). The apparently close adaptation of CyHV-3 to its host's life cycle may indicate a long evolutionary relationship (Uchii et al., 2004).

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, a review commissioned by the NCCP has recommended some further testing 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 and Holmes, 2017a).

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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 (see Section 6.2.2 for an example involving zoo animals).

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., 2012; 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).