


Review

# Biocontrol of the Common Carp (*Cyprinus carpio*) in Australia: A Review and Future Directions

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**Abstract:** Invasive pest species are recognized as one of the important drivers of reduced global biodiversity. In Australia, the 267 invasive plant, animal and microbial species, established since European colonization in the 1770s, have been unequivocally declared the most important threat to species diversity in this country. One invasive pest, the common carp (*Cyprinus carpio*), has been targeted in an integrated pest management plan that might include cyprinid herpesvirus 3 (CyHV-3) as a potential biocontrol agent. The species-specificity of the released virus (and of field variants that will inevitably arise) has been assessed, and the virus judged to be safe. It has also been hypothesised that, because the virulence of the CyHV-3 will likely decline following release, the virus should be used strategically: initially, the aim would be to markedly reduce numbers of carp in naive populations, and then some other, as yet uncertain, complementary broad-scale control measure would knock-down carp numbers even further. Brief results are included from recent studies on the modelling of release and spread of the virus, the ecological and social concerns associated with virus release, and the restoration benefits that might be expected following carp control. We conclude that, while further work is required (on the virus, the target species, environmental issues, and especially the identification of a suitable broad-scale complementary control measure), optimism must prevail in order to ensure an eventual solution to this important environmental problem.

**Keywords:** biocontrol; Australia; common carp; *Cyprinus carpio*; cyprinid herpesvirus 3; safety; efficacy; modelling; risks

## 1. Introduction

In 2013, in his comprehensive book on invasive species, Simberloff [1] suggested that biological invasions are (along with climate change and habitat destruction) one of the great anthropogenic threats to global diversity. Subsequently, a United Nations-backed panel of scientists (representing over 130 nations) produced a report in 2019 that unequivocally identified invasive species as one of the important drivers of the global decline in numbers, and frequent extinction, of native animal and plant species. The report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Sciences (IPBES) [2] noted that at least 680 species of vertebrates, alone, have been lost due to human actions taken since 1500.

The IPBES Report suggested that there were five direct drivers of reduced global biodiversity: (1) changes in the use of land and sea, (2) direct exploitation of the plants and animals of the world, (3) climate change, (4) pollution, and (5) invasive pest species. There has been a 70% increase in numbers of the latter since 1970 across 21 countries where detailed records were maintained. At about the same time that the IPBES Report was released, an Australian group declared [3] that invasive species were, in fact, the major threat to species diversity in Australia followed by modifications to ecosystems and agriculture. Furthermore, they found that this hierarchy of threats was consistent for

almost all native plants and animals in Australia, the only exceptions being native fish where pollution replaced agriculture as the third major threat.

It is estimated that, of the thousands of exotic species that have arrived since European colonization of Australia in the 1770s, 267 have become genuine invasive pest species (207 plants, 57 animals, and three microbial pathogens) [3]. Concomitant with this influx of invasive species, at least 93 native species of plants and animals have officially become extinct with the demise of many others either recognised informally, or likely to have gone unrecorded [3]. Australian inland water communities have been forced to contend with cane toads (*Rhinella marina*) and around 43 known invasive freshwater fish species including eastern gambusia (*Gambusia holbrooki*), goldfish (*Carassius auratus*) and perhaps the most disliked of all, the common carp (*Cyprinus carpio*). Surprisingly, of about 300 species of Australian freshwater fish that are recognised in 59 families, none are known to have become extinct since European colonization although there is evidence of regional extinctions, and recovery actions have probably saved several species from extinction [4]. In addition, different federal and state bodies have listed 74 freshwater species as ‘threatened’ [4]. It is likely that invasive fish species are, directly or indirectly, associated with the dire status of many Australian native freshwater fish.

*C. carpio* (known simply as ‘carp’ in Australia) was probably first introduced to waters around Sydney, Australia in 1908 (earlier records possibly being confused with goldfish, *Carassius auratus*) [5,6]. However, it was not until the 1960s that they were recognized as a serious invasive pest species, particularly in the Murray-Darling Basin (MDB), a regulated river system that covers 14% of the continent on the eastern side of the nation [4]. Carp comprise up to 90% of the fish biomass in parts of the MDB, and it is recognized that they are responsible for a deleterious cascading effect on the aquatic environment: they uproot and consume aquatic vegetation which increases the turbidity of the water. This change then leads to further reductions in aquatic vegetation, invertebrate communities, aquatic birdlife, and native fish [7].

In the early years of the new millennium, the Australian Federal Government began a program to develop innovative measures for the control of several important terrestrial and aquatic invasive pest species. An integrated pest management (IPM) plan was developed for carp in Australia, and, following the recognition of cyprinid herpesvirus 3 (CyHV-3; also known as koi herpesvirus) in Israel and the USA in 1998 [8], a research program was initiated to investigate the potential of this virus as a biological control (biocontrol) agent within the IPM plan for carp. This review summarizes the work already completed, and it also identifies the outstanding requirements before CyHV-3 could be considered as a biocontrol agent in Australia.

## 2. The Essential Information Required for Potential Viral Biocontrol of Carp

Since the 1950s, Australia’s use of two different viruses for rabbit biocontrol has demonstrated many generic lessons for future viral biocontrol programs of invasive vertebrates [9]. In broad terms, these lessons indicate the necessity for an understanding of the biology of both the targeted pest species and the putative biocontrol virus. For carp control in Australia, in particular, a vast amount of information has already accumulated on carp biology in this country, although key pieces of information were still required. However, as an exotic (or foreign animal disease) virus for Australia, specific information about CyHV-3 in Australian conditions was almost non-existent. Table 1 summarizes the essential additional information required to not only understand carp biology and ecology in this country, but also the far greater needs to provide insights into CyHV-3 activity. Much of this information on the host and the virus has now been acquired through government-sponsored research programs (“Knowns” in Table 1), but deficiencies in our knowledge still exist (“Unknowns”). Due to space considerations, the following sections briefly focus on recent additions to our knowledge about carp and CyHV-3, especially for Australia’s needs.

**Table 1.** The essential information required for potential viral biocontrol of carp.

Information Required	Knowns	Unknowns
Carp biology in Australia	<ul style="list-style-type: none"> <li>• Modelling of carp biomass across Australia in 2018</li> <li>• Future estimates of carp biomass in different hydrological scenarios</li> </ul>	<ul style="list-style-type: none"> <li>• Genomic and transcriptomic study of carp in Australia</li> </ul>
Viral epidemiology	<ul style="list-style-type: none"> <li>• Global and laboratory epidemiology</li> <li>• Genome of the virus</li> </ul>	<ul style="list-style-type: none"> <li>• Viral epidemiology in Australian conditions</li> <li>• Latency</li> </ul>
Safety of the virus(species-specificity)	<ul style="list-style-type: none"> <li>• The released virus</li> <li>• Human safety</li> <li>• Asian and North American field outbreaks</li> </ul>	
Efficacy of the virus	<ul style="list-style-type: none"> <li>• Virus-host interactions               <ul style="list-style-type: none"> <li>○ Transmission</li> <li>○ Virulence</li> </ul> </li> <li>• PCR survey of MDB carp for cyprinid herpesviruses</li> </ul>	<ul style="list-style-type: none"> <li>• Virus transmission               <ul style="list-style-type: none"> <li>○ Determine <math>R_0</math></li> </ul> </li> <li>• Virulence               <ul style="list-style-type: none"> <li>○ Virulence of different CyHV-3 isolates</li> <li>○ Virome of carp in Australia</li> <li>○ Polymorphisms in immune genes of carp in Australia</li> <li>○ Modelling of carp-goldfish hybrids in MDB</li> </ul> </li> </ul>
Epidemiological modelling of virus release and spread	<ul style="list-style-type: none"> <li>• Hydrological model</li> <li>• Habitat suitability model</li> <li>• Demographic model</li> <li>• Epidemiological model</li> </ul>	<ul style="list-style-type: none"> <li>• Validation of models</li> <li>• Extension of models across entire MDB</li> <li>• Key epidemiological rates</li> </ul>

Table 1. Cont.

Information Required	Knowns	Unknowns
Evolution of the released virus	<ul style="list-style-type: none"> <li>• Rabbit biocontrol viruses</li> <li>• Absence of native Australian cyprinids</li> <li>• Insusceptibility of most closely related fish</li> </ul>	<ul style="list-style-type: none"> <li>• Amelioration of virulence of CyHV-3?</li> </ul>
Broad- scale control measure(s) to complement the virus	<ul style="list-style-type: none"> <li>• Many regional measures available</li> <li>• Options for broad-scale measures are available</li> </ul>	<ul style="list-style-type: none"> <li>• Ideal broad-scale measure to complement the virus</li> <li>• Next generation of CyHV-3 (aquaculture isolates)</li> </ul>
Ecological concerns	<ul style="list-style-type: none"> <li>• Ecological risk assessment</li> <li>• Environmental clean-up procedures after fish kill events</li> <li>• Options for utilisation of large volumes of carp</li> </ul>	<ul style="list-style-type: none"> <li>• Prey switching</li> <li>• Viral epidemiology in Australian conditions</li> </ul>
Social risks	<ul style="list-style-type: none"> <li>• Views of urban versus MDB populations on CyHV-3 and biocontrol</li> </ul>	<ul style="list-style-type: none"> <li>• Views of urban versus MDB populations post-release of CyHV-3</li> </ul>
Restoration benefits from carp control	<ul style="list-style-type: none"> <li>• Expert elicitation study on the ecological consequences of reduced carp numbers</li> </ul>	<ul style="list-style-type: none"> <li>• Control of other environmental stressors</li> </ul>

## 2.1. Carp Biology in Australia

### 2.1.1. Distribution Models of Carp in Australia and Biomass Estimates

A great deal of excellent work has been conducted on carp biology since the 1960s when the species was first recognized as an important pest in Australian waterways; as examples, see [7,10–13]. However, while estimates of carp biomass in the MDB have been proffered in the past, any national carp biocontrol program would require a modern, more extensive and more accurate approximation. Working across numerous jurisdictions, Stuart et al. [14] used catch-based models to generate “heat maps” that depicted the biomass and spatial distribution of carp throughout the waterways of south-eastern Australia in 2011 and 2018. It was already known that when carp exceed a threshold density of 80–100 kg/ha, detrimental ecological impacts may occur [11]. Stuart et al. [14] found that modelled carp biomass exceeds this threshold across large areas of south-eastern Australia and therefore is consistent with the view that carp may have landscape-scale impacts manifested by the decline of water quality, native flora, fauna biodiversity, and recreational values. In short, carp represent a serious threat to freshwater ecosystems.

The 2011 and 2018 biomass estimates [14] were based on static spatial mapping. However, because carp populations can respond rapidly to changes in hydrological conditions, these estimates cannot be applied to future scenarios when a biocontrol virus might be released. Therefore, Stuart et al. [14] recommended the use of a dynamic model to provide future estimates of carp biomass, taking into account a variety of possible hydrological scenarios. Todd et al. [15] undertook dynamic modelling using an established carp population model [13] and the static biomass estimate for 2018 [14]. They then provided a range of estimates for the biomass of carp for 2023 in four regions of south-eastern Australia. Their results highlighted the variability of populations with differing hydrological and ecological conditions, and this, in turn, emphasized the advantage of dynamic modelling: it provides managers with a current estimate of carp populations in different locations, which then assists managers when considering where to release a biocontrol virus, and also where to focus clean-up operations to ameliorate the impacts of large numbers of dead carp.

### 2.1.2. Genomic and Transcriptomic Map of Carp in Australia

Using variability in 14 microsatellite loci, Haynes et al. [5] studied the population genetics of carp at each of 34 locations throughout the MDB. They confirmed the presence of the four recognized strains in Australia: Boolarra, Yanco, koi and Prospect, despite Prospect being originally restricted to Sydney (outside of the MDB). They also concluded that there was significant genetic structuring of carp that was associated with barriers to dispersal. In fact, they divided the MDB into 15 management units, each unit based on man-made or natural barriers to dispersal of carp. They noted that, while invasive species often show decreased levels of genetic diversity in a new location, some actually have similar, or greater, diversity due to the invasives being introduced a number of times from different sources. This apparently applies to carp in the MDB which have high levels of genetic diversity (with multiple strains in all regions). They warned that the 15 management units should be interpreted with caution because fish-ladders may increase connectivity.

While the work of Haynes et al. [5] has been valuable in providing a preliminary understanding of the population genetics of carp in Australia, there is a dire need for a genomic study of this pest species in the MDB. This would provide a level of information on the targeted pest that is commensurate with our knowledge of the Indonesian strain of CyHV-3 that has been identified as a biocontrol virus in Australia (see Section 2.2). There are at least two immediate needs that could be addressed by a genomic study of carp in Australia: information on the virome of carp in this country, and an understanding of the polymorphisms in some immune response genes that may be critical in determining the virulence of CyHV-3 (see Section 2.4).

## 2.2. Viral Epidemiology

Since 1998, virulent CyHV-3 has been identified in many countries, but there is no evidence for its presence in Australia [16]. Although the origin of the virus remains problematic, it appears to have arisen in recent decades, possibly from avirulent variants in Europe [17], but there is also evidence for unusual variants in New York State [18] and Oregon [19] in the USA. Other molecular studies [20] also support the idea that an avirulent variant(s) of CyHV-3 has been present in *C. carpio* for tens of thousands of years although Kopf et al. [21] highlighted two major assumptions that perhaps cast some doubt on this time-frame—firstly, that the evolutionary rate of CyHV-3 has been constant, and secondly, that this rate for an alloherpesvirus from an exothermic host is similar to an alphaherpesvirus from an endotherm.

Under permissive conditions, CyHV-3 can cause 70–100% mortality in juvenile and adult *C. carpio* [22–24]. However, larvae less than 1 cm in length are completely resistant to infection due to the protective effect of skin mucus. Larvae gradually become susceptible with increasing size, culminating in complete susceptibility when they are longer than 2 cm [25–27]. Only very low doses of virus are required for infection [28], the main portal for both infection and excretion being the skin [29]. Once excreted from an infected fish, a virus survives in the aquatic environment for only about three days, regardless of water temperature [30]. While most of these epidemiological data have been acquired from overseas studies, there is little reason to expect major differences under Australian conditions. However, due to biosecurity concerns with CyHV-3, this view has not been proven because field trials are yet to be conducted in this country.

An Indonesian strain of CyHV-3 (the C07 isolate) will potentially be used as the biocontrol virus in Australia. The full genome sequence has been determined [31], revealing that the gene layout is very similar to CyHV-3-U (a US reference genome) although 310 genetic variations between the C07 strain and the reference genome were identified. Phylogenetic analysis inferred from comparisons of whole-genome sequences revealed that the Indonesian isolate is more closely related to a Japanese isolate within the Asian lineage than to isolates within the European lineage.

Being a herpesvirus, CyHV-3 is likely capable of inducing latent infections in surviving carp, but while initial studies have demonstrated that low-temperature persistent infections are possible [32,33], unequivocal latent infections are yet to be demonstrated [34].

## 2.3. Safety of the Virus

Two of the most important lessons from Australia's earlier work on rabbit viral biocontrol have been the necessity for assessing both the safety and efficacy of any potential biocontrol virus. 'Safety' is about species-specificity, not only of the virus isolate selected for potential release into the environment, but also of any future generations of the virus that may evolve genetic changes (mutations or recombination) following release in the field (see Section 2.6).

The most compelling evidence for the specificity of CyHV-3 is that viral-induced disease has never been reported anywhere in the world in any species other than *C. carpio* since CyHV-3 was first recognized. This includes species in polyculture systems with carp. It is an observation that has often been ignored by critics of the virus, but its importance should never be overlooked. Importantly, this broad observation includes humans whose fears of infection can also be allayed by several observations: there has been no evidence of adverse effects on humans working in CyHV-3-affected carp farms. The two closely-related viruses, CyHV-1 and -2, are not known to infect humans. There was no evidence of infection in CyHV-3-challenged mice (selected as a representative mammal in non-target species susceptibility trials) [16]. More generally, there is no evidence for any fish virus causing disease in humans [35]. These findings were corroborated by Roper and Ford [36] who, in addition, recommended that the "psychosocial effects" on human health of a mass fish kill should be investigated.

For other potential targets, numerous laboratories have suggested that many species could become infected by CyHV-3, but without causing disease. In summary, the susceptibility of 24 to 25 species of

fish was tested [22,37–42], and, in all cases, there was no evidence of disease. While CyHV-3 genomic DNA was detected in 10 of 15 species of fish that were exposed to acutely- or latently-infected carp, only a small proportion of each species was supposedly infected, none showed clinical signs of disease, and only low copy numbers of CyHV-3 DNA were found [39]. Similar results were found for plankton, mussels and crustaceans [43,44]. In none of these cases, however, was there an attempt to demonstrate CyHV-3 mRNA as an indicator of virus replication, a necessary corollary of infection. It should be noted that even though one study [45] did use an RT-PCR, ostensibly to demonstrate replication of CyHV-3, their work was flawed technically in that neither primer in their RT-PCR was designed in separate viral exons nor over splice junctions. It is likely that their primers were actually detecting residual contaminating genomic DNA from virus rather than viral mRNA [16].

Yuasa et al. [46] eventually developed an RT-PCR for CyHV-3 that allowed differentiation of genomic DNA from the mRNA of replicating virus. This allowed a definitive laboratory study on the susceptibility of the following non-target species (NTS) to CyHV-3 [16]: 13 native Australian fish species, introduced rainbow trout (*Oncorhynchus mykiss*), native lamprey ammocoetes (*Mordacia mordax*), domestic chickens (*Gallus gallus domesticus*), laboratory mice (*Mus musculus*), a freshwater crustacean (*Cherax destructor*), two species of frogs (*Litoria peronii* and *Lymnodynastes tasmaniensis*), and two reptilian species (*Intellagama lesueurii* and *Emydura macquarii*). When challenging each of these NTS, CyHV-3 was given the best chance of causing disease through the use of immature, susceptible NTS that were exposed, by immersion and/or intraperitoneal inoculation to 100–1000 times the dose of virus required to infect a carp.

All challenged NTS were subjected to clinical, gross pathological and histopathological examinations, and to PCR testing (using a screening qPCR, and the specific RT-PCR [46] to re-examine any qPCR-positive samples). While low copy numbers of CyHV-3 DNA were found in occasional samples by qPCR, all such samples were negative for viral mRNA by the RT-PCR suggesting that the weakly-positive qPCR results were, in fact, due to low-level contamination events during processing of samples rather than to the presence of replicating virus. Thus, it was concluded that no evidence could be found for infection, let alone disease, in any of the NTS. Boutier et al. [47], however, offered alternative interpretations. Firstly, they suggested that “technical issues” were not addressed (although they provided no specific details on what these issues might be), and, secondly, that the deaths in NTS could have been due to a non-replicative pathogenesis such as may occur in herpesvirus latent infections of non-natural host species [48]. The latter is an interesting suggestion, but seems to overlook two important observations: (1) latency, although likely to occur in carp surviving infection with CyHV-3, has not actually been demonstrated yet in the host species, let alone a NTS [34], and (2) a productive infection, the necessary precursor to a latent infection, has not even been demonstrated in any NTS. For example, McColl et al. [16] did not find clinical signs of disease, histological lesions or any evidence of an early productive infection (in the form of viral transcripts) in any of their NTS inoculated with CyHV-3 despite examining many NTS at early and later stages following inoculation.

Kopf et al. [21], while accepting that adverse effects of the virus on native species are “highly improbable”, were, however, still loathe to absolve CyHV-3 of all potential threat. They suggested that native species could be “asymptomatic carrier(s)” or transmitters of the virus. Again, this ignores the fact that to be a carrier, a non-target native species must first be infected, a claim that has never been properly demonstrated (see above). Furthermore, claims for all but very short-term transmission by non-carp species were refuted by the elegance of the simple experiments with CyHV-3 on goldfish [49]. The final argument by Kopf et al. [21], that sub-lethal infections in immunocompromised NTS be investigated, has, indirectly, already been addressed by McColl et al. [16] through the use of immature fish (with incompletely developed immune systems) in their susceptibility studies on NTS.

In summary, we believe that a robust standard protocol is required for future susceptibility testing of NTS, and we propose the following: (1) time-course sampling to demonstrate an increase or decrease of viral DNA concentration in a viral-exposed NTS, (2) using both qPCR and RT-qPCR for detecting viral DNA and mRNA, respectively, (3) attempting virus isolation from any NTS with clinical signs of



disease, and (4) using histopathological examination on moribund NTS. Molecular testing, including next generation sequencing if available, should also be considered for exclusion of other known or unknown pathogens.

The results of the NTS experimental work [16] were complemented by the findings from a North American study of natural outbreaks of CyHV-3 in carp [50]. At each outbreak, no disease was observed in any co-habiting species, even in native cyprinids, thus attesting to the species-specificity of the virus. However, there are two important criticisms of other observations in the North American work: (1) there was no attempt to look for the presence of any pre-existing, potentially cross-reactive viruses that might confer protection on carp. In particular, there have been no reported serological, PCR or next-generation sequencing studies on any carp populations in North America, and (2) only one of the outbreak sites offered the opportunity for direct fish-to-fish transmission by means of dense aggregates of carp. In the Thresher et al. study [50], there appeared to be few, if any, equivalents of the limited numbers of densely populated carp breeding sites distributed throughout the MDB. Thresher et al. [50] also claimed, probably correctly, that mass die-offs would not be expected for a herpesvirus that is in equilibrium with its natural host. CyHV-3, however, is a pathogen that has only recently been recognised [8], possibly because it has only recently arisen [20]. Therefore, it has not yet had time to come into equilibrium with its host, in which case, high mortalities are probably not unexpected.

#### 2.4. Efficacy of the Virus

Determining the ‘efficacy’ of a potential biocontrol virus is slightly more complex than determining its ‘safety’ because the former depends on two variables, ‘transmissibility’ and ‘virulence’. For a biocontrol virus, ‘transmissibility’ is defined as the ability of the virus to establish infection in new hosts, and is often measured by the basic reproduction number,  $R_0$ , the average expected number of cases produced by a single case (in a population where all individuals are fully susceptible). ‘Virulence’ is a measure of the severity of the disease caused by the virus, not necessarily measured simply by mortality. For example, in the classical studies of the MYXV, Fenner and Woodroffe [51] established five grades of virulence that were based on a combination of both survival time following infection, and mortality.

Using Australia’s two rabbit biocontrol viruses as examples, Di Giallonardo and Holmes [52] demonstrated that, while there are invariably strong selection pressures for transmissibility, this has been achieved for MYXV and RHDV by selection in the field of virus strains of intermediate and high virulence, respectively. These quite different paths suggest that, for any particular virus, it is not always easy to predict the outcome of the complex relationship between transmissibility and virulence. There have been no direct studies on how CyHV-3 achieves maximal transmission, but observations on viral epidemiology, particularly the virus–host interaction, may encourage the formulation of two hypotheses [53].

Firstly, the observations that CyHV-3 is excreted at low titre into an aquatic environment, and then only survives for about three days outside its host [30,54], suggest that direct transmission of virus between carp is likely to be much more important than indirect transmission via the aquatic environment. Furthermore, given that carp are highly sensitive to infection [28], and that the skin is the main portal of both infection and excretion of CyHV-3 [29], a reasonable hypothesis is that direct skin to skin contact between an infected and an uninfected fish, even if transient, is the most likely form of transmission. Such contact would likely disrupt the skin mucus layer which would enhance virus entry [26,27]. Clearly, carefully designed transmission experiments are required to test this hypothesis.

Having become infected, a viraemia develops in the carp, and the virus localizes in various tissues [55]. The adaptive immune response of the fish develops slowly [56–58], likely allowing survival of some infected hosts with potential latent infections although, as already mentioned, latency has not yet been proven unequivocally [34]. Nevertheless, assuming it does indeed occur in surviving fish (as it does in the hosts of all known herpesviruses), then recrudescence of acute



infections will also occur during periods when infected fish are stressed. In Australia, massive aggregations of carp occur at annual breeding events, and such aggregations are known to induce stress and immunosuppression [59]. This, in turn, implies that annual breeding would not only allow reactivation of CyHV-3 infections, but would also favour transmission of virus by direct skin-to-skin contact of the densely aggregated fish. These observations then suggest a second hypothesis: that long-term transmission of CyHV-3 in Australian conditions may be favoured by the natural selection of low virulence strains of CyHV-3 that would allow survival of some latently-infected fish which, in turn, would lead to multiple periods of recrudescence and transmission of virus to naive fish during annual breeding events. It was postulated earlier that selection pressures may change as the density of carp declines [53], but, on reflection, this possibility may be of little importance if, indeed, most transmission occurs at densely aggregated breeding sites. The latter sites will likely form regardless of the total number of carp in the river systems because, at least in Australia, carp seem to be irresistibly attracted to these sites at certain times of the year [60]. So, while the total area of any particular breeding site may decline, the density of fish will probably remain high.

A legitimate question that arises because of the second hypothesis is that, if transmissibility drives the selection of low virulence strains of CyHV-3, can the virus be an effective biocontrol agent in Australian waters? Perhaps the answer may be found in lessons from past viral biocontrol programs involving rabbits in Australia [9]. Field experience with both MYXV and RHDV has revealed that a virus, alone, will not control the targeted invasive pest species. In fact, to be effective, biocontrol viruses must be complemented by other broad-scale control measures, a fact that has been emphasized many times from the outset of the carp biocontrol program in Australia. The use of such measures is not an admission of failure in the proposal to use CyHV-3 as a biocontrol agent; rather, it is an argument for the use of the virus in a carefully designed IPM program. The virus would markedly reduce numbers of carp in naive populations, providing the opportunity for complementary measures to then substantially knock down carp numbers even further (see Section 2.7).

The second factor affecting the efficacy of the virus, virulence, may be difficult to determine. While Fenner and Woodroffe [51] used standard inbred laboratory rabbits for their work on the virulence of MYXV, no equivalent line of carp is available in Australia to allow a standard test of the virulence of different isolates of CyHV-3, nor, indeed, to determine  $R_0$ . However, the virulence of the C07 isolate of CyHV-3 has been demonstrated in numerous studies on carp collected from all over south-eastern Australia for example, [16,61,62]. Further studies are required to test the virulence on carp collected from throughout the entire MDB.

Boutier et al. [47] expressed a number of reservations about the use of CyHV-3 as a biocontrol agent for carp in Australia. They contended that natural resistance of some carp (due to resistance-conferring polymorphisms in immune genes) and of carp-goldfish hybrids could lead to rapid proliferation of resistant phenotypes. Access to genomic and transcriptomic maps of Australian strains of carp would help to address the question of immune genes, while assessing the future importance of carp-goldfish hybrids would likely require modelling work and a better understanding of the current numbers of these hybrids in the MDB.

Boutier et al. [47] also proposed that phylogenetic studies suggested that CyHV-3 may already be present in carp in Australia, just as it may have long been present, without expressing virulence, in carp populations around the world [20]. Studies on 849 carp samples from nine sites throughout the MDB in Australia, utilizing a nested PCR (with primary and nested primers aligning perfectly with sites in the DNA polymerase gene of CyHV-1, -2, and -3), failed to reveal evidence for any known or undescribed cyprinid herpesviruses [63]. Nevertheless, this is recognized as only a preliminary study, and a more definitive virome study (using a next generation sequencing approach) from a similar sample of carp is essential to corroborate the PCR work.

Finally, in assessing the likely efficacy of CyHV-3 in Australia compared with natural overseas outbreaks [21], it is important to recognize a critical difference between the two situations: whereas most of the world is consumed with controlling outbreaks of CyHV-3 disease, Australia would aim to

enhance the spread of the disease (and then to augment the effect of the virus with complementary control measures). To this end, it is essential that we have a deep understanding of the epidemiology of the disease under Australian conditions. Kopf et al. [21] state that “Lake Biwa (in Japan) and Blue Springs Lake (in the USA) are not good models for Australian conditions if the virus was (sic) released”, but, nevertheless, most of their criticism of Australian activities is based on findings from these overseas outbreaks. Based on the known biology of the virus and of carp in Australia, we have proposed two hypotheses that account for virus pathogenesis and transmission under Australian conditions; aspects of these hypotheses have informed the work on epidemiological modelling.

### 2.5. Epidemiological Modelling of Virus Release and Spread

A large multi-disciplinary team developed four inter-related models, namely hydrological, habitat suitability, carp demographic, and epidemiological models, with the intention of informing any future staged release of CyHV-3 in the MDB [64].

The hydrological model focussed on the water temperature and connectivity of waterways for five diverse catchments in south-eastern Australia. It concluded that, while CyHV-3 will generally be effective from Spring through Autumn throughout south-eastern Australia, a staged release of virus would demand precise estimations of water temperature prior to release in any particular catchment to ensure conditions were permissive for virus activity. However, water temperature alone was insufficient for determining the time of virus release. It was also found that the major environmental factors influencing the distribution and abundance of carp in south-eastern Australia, and the manner in which these factors interacted with each other, were also essential in selecting a time for virus release.

This conclusion was reached through a habitat suitability workshop that utilized expert opinion within the context of a Bayesian belief network (BBN). The BBN identified river flow and water temperature as the two essential parameters determining the suitability of a habitat for adult and sub-adult carp, and both were rated as medium to high for most habitats throughout the study period. On the other hand, waterway inundation and connectivity, the essential habitat suitability factors for an abundance of larvae and young-of-year (YOY) stages, were rated poorly in most habitats during the study period. Population abundance for YOY stages, in fact, depended on a relatively small number of dense aggregations of juveniles and adults that occur in transiently flooded wetlands throughout the MDB (so-called ‘recruitment hotspots’). Through the use of conversion factors guided by expert opinion, habitat suitability rankings were converted to biomass density estimates (the latter validated by recently acquired data [14]). These estimates would then allow CyHV-3 to be used in those areas where the population density of carp was approximately 80–100 kg/ha, the level at which detrimental ecological impacts may occur [11].

The biomass densities from the habitat suitability model were then used to develop a full spatio-temporal population projection (or demographic) model of carp population dynamics in which carp metapopulations were resolved into six age-stage classes (eggs, larvae, early YOY, late YOY, sub-adults, and adults). This demographic model, in turn, was integrated into a CyHV-3 epidemiological model that allowed the prediction of mortality and suppression of the subpopulations following a hypothetical release.

The epidemiological model was a variation on the standard SEIR transmission model. It included susceptible (S), exposed (E) and infectious (I) classes, but the usual recovered (R) class was replaced with classes more likely to represent a typical herpesvirus, such as CyHV-3. Thus, latent (L) and recrudescence (Z) classes were introduced, with Z representing second and subsequent infections following repeated reactivation of the virus in latently infected carp. The modelling predicted that, without recrudescence, introduction of the virus would be associated with a single mortality event in carp. However, in the more likely event of latency and recrudescence, there would be an ongoing and lasting suppression of carp populations in all catchments with reductions being to approximately 40% of the pre-release population. The impact of the virus would be sufficient to reduce carp populations in many MDB waterways to below the damage threshold of 100 kg/ha for at least 10 years. A further notable prediction

was that seasonal losses would be mainly in immature carp, the mortality in the adult population being less by at least an order of magnitude (and, therefore, possibly not easily observed).

Boutier et al. [47] suggested that, at various times of the year, there could be vast tracts of water in the MDB where temperatures may be non-permissive for virus replication. These concerns have long been noted [9]. However, the modelling report [64] specifically noted that “tailoring the release of the virus to the particularities of each catchment” would be important, especially in those areas where there was a very narrow window of permissive Spring temperatures. The use of other complementary control measures (see Section 2.7) could also be important in some areas. Boutier et al. [47] also suggested the importance of ‘behavioural fever’ in fish as a response to infection. There is no question that this phenomenon works for individual fish, but it has not prevented mass mortalities of carp in thermally variable natural aquatic environments overseas, and it would be unlikely to do so in Australia either (particularly if a virus were to be released in relatively homogeneous shallow breeding grounds of carp).

## 2.6. Evolution of the Released Virus

While laboratory work and field observations strongly suggest that current strains of CyHV-3 are highly specific for *C. carpio* (and some hybrids [65,66]; see Section 2.3), the question remains about the likelihood of genetic changes in current field isolates causing a future expansion of the host range. There is no direct evidence bearing on this question for CyHV-3, but there are pertinent lessons from Australia’s past experience with viral biocontrol of rabbits. Evolutionary studies [67,68] have shown that DNA viruses can, indeed, mutate (although at a much lower rate than RNA viruses). However, while this may potentially allow spill-over events or host-jumps, such events for herpesviruses occur on timescales of millions of years, and, when they occur, they are invariably into taxonomically closely related species. It is reassuring then that, while there are a number of introduced cyprinids in Australia, there are no native cyprinids. Furthermore, the most closely related native Australian species (native catfish) are insusceptible to infection with CyHV-3 [16], and, again, it should be emphasized that viral-induced disease has never been reported anywhere in the world in any species other than *C. carpio*.

Field observations on rabbit biocontrol viruses in Australia lend support to these evolutionary studies [9]. Mutations are known to have occurred in the field in both the myxoma virus (MYXV, a DNA virus present in Australia for over 60 years) and in rabbit haemorrhagic disease virus (RHDV, an RNA virus, over 20 years) [69,70], but there is no evidence that either has jumped into another species. As noted by Di Giallonardo and Holmes [71], the overall conclusion is that “host-jumps to nontarget species are not an inevitable consequence of viral evolution”. As a result, all observations, whether on current isolates or potential future field variants of CyHV-3, encourage the view that the chance of cross-species transmission is very small.

## 2.7. Broad-Scale Control Measure(s) to Complement the Virus

Saunders et al. [72] found that there have only been three major instances where viral pathogens have been used successfully against vertebrate pest species, namely MYXV and RHDV against rabbits in Australia and feline panleukopenia virus against cats on a South African offshore island. In reviewing the lessons from these attempts at viral biocontrol of invasive vertebrates [9], it was noted that in each case complementary measures were required for sustained control or eradication of the pest species. While these measures actually included supplementary regional controls, ideally broad-scale controls would be identified and implemented. A number of regional control measures have long been implemented for carp, including commercial harvesting, electrofishing, carp traps, fishing competitions, predator stocking, poisoning, and environmental controls [7,73,74]. However, these are generally ineffectual in the long-term.

The development of broad-scale control strategies that will deliver persisting declines in carp numbers has been more problematic. Wedekind [75] reviewed possible genetic biocontrol technologies that could be used on carp populations in Australia. He broadly classified them as those that involve genetic engineering (including ‘daughterless’ carp and gene-drive technologies) and those that do not (including ‘Trojan Y chromosome’ techniques).

Of the techniques involving genetic engineering, an early major investment was made into ‘daughterless’ carp technology in Australia [76]. The underlying principle was that, by using an RNAi approach that suppressed expression of the female differentiating genes, natural carp populations in Australia would be biased towards all-male populations. Although initially very promising, the approach gradually fell out of favour because modelling revealed that it would take many decades to exert an impact, the corollary being that very large numbers of these genetically modified fish would need to be added to waterways annually for many years in order to force a sex bias in natural populations. Currently, a gene-drive approach that would be lethal to female offspring, or leave them infertile, is not considered a safe option for carp [75], nor indeed for any biocontrol program [77]. In the future, however, gene-drive technology may become a universal approach to controlling invasive pest species, although many modifications to current technology will be required for this approach to become acceptable.

Approaches that do not involve genetic engineering include the use of Trojan Y fish [78]. This method relies on treating young male carp with a female sex hormone, oestrogen, resulting in genetic males (with XY chromosomes) that have female sex characteristics, including the ability to produce eggs. The latter have an XY constitution, and when fertilized by a normal male, they produce a preponderance of male offspring. Extensions of this basic approach can lead to stock populations of YY individuals being produced for release into wild populations [79]. Although this strategy would require the costly regular addition of modified fish to natural populations of carp for a number of decades, it is considered the most appropriate current technique, particularly if combined with measures to increase the survival and fecundity of the manipulated carp [75].

In the immediate future, perhaps new, more virulent strains of CyHV-3 may prove to be a useful complementary measure. A situation may develop that is analogous to the commercial chicken industries where the herpesvirus, Marek’s disease virus (MDV), has been an ongoing threat for many decades. Strains of MDV of increasing virulence have evolved due to the use of imperfectly immunizing vaccines. Similar vaccines have been used in carp aquaculture to protect farmed carp from outbreaks of CyHV-3 for about a decade, and it is hypothesised that they too may lead to the evolution of more virulent strains of CyHV-3 that could be used as the next-generation biocontrol viruses in Australia [53].

In summary, a number of options for a broad-scale complementary control exist (Table 2). Each has their strengths and weaknesses, but currently, we are in complete agreement with Boutier et al. [47] and Kopf et al. [21] in declaring that the ideal broad-scale complementary measure(s) has not yet been identified. However, as previously mentioned [34], new genetic options continue to appear [80], providing optimism that the ideal measure will soon be developed. Until then, it would be unwise, even wasteful, to release CyHV-3 into the Australian environment.

**Table 2.** Broad-scale control options to complement CyHV-3 biocontrol of carp in Australia.

Technology	Strengths	Weaknesses	Comments
Trojan Y chromosome	<ul style="list-style-type: none"> <li>Fish are not GMO</li> <li>Technologies to produce Trojan Y carp are available</li> <li>Very useful if current carp numbers reduced by CyHV-3</li> </ul>	<ul style="list-style-type: none"> <li>Need regular releases of treated fish</li> <li>Decades to achieve major reduction in numbers (if used alone)</li> </ul>	<ul style="list-style-type: none"> <li>Already well-developed technology</li> <li>Should combine with measures to increase the survival and fecundity of Trojan Y carp</li> </ul>
Daughterless carp	<ul style="list-style-type: none"> <li>Very useful if current carp numbers reduced by CyHV-3</li> </ul>	<ul style="list-style-type: none"> <li>GMO fish</li> <li>Need regular releases of treated fish</li> <li>Decades to achieve major reduction in numbers (if used alone)</li> </ul>	<ul style="list-style-type: none"> <li>Already well-developed technology</li> </ul>
Gene-drive	<ul style="list-style-type: none"> <li>Very few carp need to be released to affect the population</li> <li>Very useful if current carp numbers reduced by CyHV-3</li> </ul>	<ul style="list-style-type: none"> <li>GMO fish</li> <li>Potentially difficult to contain or reverse in case of unexpected outcomes</li> </ul>	<ul style="list-style-type: none"> <li>Need to identify germline (sex) specific promoters in carp</li> <li>Other potential technical problems</li> </ul>
Self-stocking incompatible-male system (see [78])	<ul style="list-style-type: none"> <li>System is both self-amplifying and self-limited.</li> <li>Control achieved with low biomass</li> <li>Reduced production overhead</li> <li>Potential applications include carp elimination or prophylactic barrier.</li> </ul>	<ul style="list-style-type: none"> <li>GMO fish</li> <li>Stocking rate high compared with gene-drive, but may be lower than other options</li> </ul>	<ul style="list-style-type: none"> <li>Technology that will likely be applicable to other invasive species</li> <li>Potential technical problems</li> </ul>
More virulent strain of CyHV-3	<ul style="list-style-type: none"> <li>Virus is not GMO</li> <li>Marek's disease precedent suggests that vaccination of carp in aquaculture will generate more virulent strains of CyHV-3</li> </ul>	<ul style="list-style-type: none"> <li>Identifying more virulent strains will likely be an ongoing process</li> </ul>	<ul style="list-style-type: none"> <li>More virulent strains of virus as a result of long-term vaccination programmes</li> </ul>



## 2.8. Ecological Concerns

Kopf et al. [21] raised the possibility of “broad ecological risks of unintended and perverse outcomes from biocontrol with CyHV-3”. They suggested a number of potential problems that could arise as a result of mass mortality, and subsequent decomposition, of carp following release of virus. Australia’s National Carp Control Program [81] undertook a number of studies to address such concerns.

Beckett et al. [82] conducted a comprehensive ecological risk assessment of the consequences of the proposed release of CyHV-3 in a variety of aquatic settings including transient wetlands, river systems, lakes and other water bodies. Impacts on water quality were considered most likely in locations characterised by a high carp biomass and low water flow such as occurs during carp breeding in transient wetlands. It was suggested that risks to native fish and birds, in particular, could be avoided by releasing the virus during high-flow seasons, or by the partial removal of carp from waterways prior to the release of virus. Whether this would be a practical option for the release strategy would need to be considered. If not, then mitigation strategies might include physical removal of carp carcasses from affected areas, or the use of water regulation to flush not only carcasses but also cyanobacterial blooms from affected areas. These strategies would likely also reduce the risk of outbreaks of botulism in native species, although both a literature review and field experience in south-eastern Australia (where there have been many fish kills due to blackwater events) suggest that botulism is unlikely to have much practical importance anyway. The loss of many juvenile carp from wetlands treated with CyHV-3 raised the spectre of prey-switching by piscivorous waterbirds. While the potential impact on native fish and other species must certainly be considered, insufficient research has been conducted in Australia to allow firm conclusions on the many potential interactions. It is, however, noteworthy that a number of earlier studies on prey-switching have revealed complex dynamics between predators and prey species, but little cause for long-term concern about the survival of native species [83–85].

Finally, Beckett et al. [82] noted that residual uncertainty necessarily remains because it is not possible to predict, with confidence, the epidemiology of CyHV-3 in Australia. The extent of carp mortality, more or less than predicted, is a key uncertainty. Similarly, the impacts of low dissolved oxygen levels (DO) on the many and varied native aquatic species can never be certain, although water quality modelling studies [86] suggested that dangerously low DO was only likely to be a problem where carp biomass was high and there was a concomitant severely compromised (or absent) water flow. A similar situation is predicted for widespread cyanobacterial blooms.

In broad terms, other very important ecological considerations are, firstly, the clean-up procedures for carp following a mass mortality due to CyHV-3, and, secondly, the potential waste utilisation of the subsequent large masses of dead carp. A literature review revealed very limited information about clean-up processes following a fish-kill [87], and therefore, not surprisingly, most of the documented responses were of a reactive nature. However, the Atlantic region of Canada is one of the few locations that does have a well-documented clean-up procedure. Silva et al. [87] declared that the biomass of carcasses and the location of the fish-kill should be important determinants, among others, of the extent of the clean-up operation, particularly if the affected waterway is part of a town water supply (in which case the suggested importance of a cost-benefit analysis for the operation almost seems paradoxical). Globally, most fish-kills have relied on landfills for the disposal of carcasses.

Tilley et al. [88] investigated alternative methods of disposal, and found that composting methods, that are able to use even severely degraded material, are likely to be the best option on a large commercial scale. However, flexibility and scalability of the process would also allow small scale operations in remote regions up to larger scale operations by councils or smaller commercial organizations. A large-scale rendering option at a meat rendering facility was also shown to be possible, although only fish carcasses < 24 h post-mortality would be acceptable for processing.



### 2.9. Social Risks

Zhang et al. [89] undertook a risk assessment to determine public perceptions about the ecological and social risks associated with the proposed use of CyHV-3 as a biocontrol agent. They conducted wide-reaching qualitative and quantitative surveys of the Australian public, focussing in the former on the general public, and in the latter on those in urban settings versus those living near major waterways. An important finding was that people who live in the MDB and who are closely connected to the river system were more likely to accept the need for carp control while still retaining some reservations about aspects of the process. Overall, the studies emphasized the importance of early, effective communication programs in order to allay the concerns of various communities.

### 2.10. Restoration Benefits from Carp Control

Finally, Kopf et al. [21] questioned whether Australia could expect to see any ecological restoration benefits from carp control. Casual observation of affected waterways has long suggested that carp must be exerting a profound negative ecological impact, especially in those regions of the MDB where they account for up to 90% of the biomass.

However, the very comprehensive study conducted by Nichols et al. [90] relied on more than casual observation. Assuming the proposed biocontrol program would be successful in reducing carp numbers, an expert elicitation study was conducted on the expected medium- (5–10 years) to long-term (beyond 10 years) ecological consequences of a reduced carp population in Australia. The study addressed the effects on ecosystems as a whole, along with effects on the animal and plant components, and, on water quality.

In summary, the study found that Australia's waterways are complex ecological systems, and they will almost certainly continue to degrade if nothing is done to control carp. On the other hand, if carp populations could be sustainably reduced by 70–100%, experts believe there would likely be clear long-term ecosystem benefits [90]. The same experts also emphasised that carp are not the only ecological stressor, and that other widespread environmental problems must also be addressed. However, even under ideal conditions where all stressors are identified and controlled, the experts agreed that, rather than restoration of a degraded system to its original state, an unexpected new aquatic ecosystem may be generated.

## 3. Final Comments and Conclusions

Australia's experience with MYXV and RHDV for rabbit biocontrol has taught us a great deal about the principles of viral biocontrol for any invasive pest vertebrate species. Perhaps the most important lessons are that it is essential to have a deep understanding of the biology of both the targeted pest species and any potential biocontrol virus. Equally important is the lesson that a viral biocontrol agent, alone, can never be expected to completely eradicate an invasive pest species; to be successful, biocontrol agents must be complemented by other broad-scale control measures in an integrated pest management program.

Is there a need for a contingency plan for any proposed biocontrol virus in the event that, following release, it fails to work as expected? Assuming all the necessary precautionary studies have been conducted, particularly on the safety, efficacy, and epidemiology of the virus prior to its release, then the most likely reason it would be judged a 'failure' is that, after an initial burst of mortality, it apparently became ineffectual. This was the experience with MYXV in Australia in the 1950s. However, in later years, it was shown that, even as rabbit mortality due to MYXV declined, the virus still held rabbit numbers below the level at which they caused environmental damage [72]. Then, with the advent of effective complementary control measures, there has been sustainable control of rabbits for approximately 60 years in Australia, and, even in the absence of eradication, biocontrol of rabbits has delivered significant economic benefits to Australia [91]. Assuming that appropriate

additional controls are found to complement CyHV-3, we would anticipate a similar trajectory for carp control in Australia.

Our world currently faces myriad local and global challenges including, but not restricted to, climate change, overpopulation and loss of species biodiversity. In Australia, the control of invasive pest species, including carp, sits comfortably in this list of challenges. We need to do something about carp to improve the quality of our waterways in this country, and it is only reasonable and rational debate that will form the foundation of future decisions and actions. CyHV-3 appears to be a rare opportunity to control carp, although, as mentioned earlier, we recognise the importance of identifying a broad-scale control measure to complement the future activity of the virus in Australia. To release the virus prior to implementation of such a complementary measure would be very unwise.

However, placing a temporary embargo on the use of the virus is not to endorse inactivity. As a marine biologist recently noted in a general commentary, “unrelenting doom and gloom in the absence of solutions is not effective. Social scientists have known for decades that large problems without solutions lead to apathy, not action” [92]. We must all recognise our current progress and successes so that in 5–10 years we can all take pride in the contribution we made to carp control in Australia.

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