TECHNICAL PAPER 2

NATIONAL CARP CONTROL PLAN

Epidemiology and release strategies

This suite of documents contains those listed below.

NCCP TECHNICAL PAPERS

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2. Epidemiology and release strategies
3. Carp biocontrol and water quality
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Will carp virus biocontrol be effective?

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4. 2016-170: Development of hydrological, ecological and epidemiological modelling
5. 2017-135: Essential studies on Cyprinid herpesvirus 3 (CyHV-3) prior to release of the virus in Australian waters
6. 2020-104: Evaluating the role of direct fish-to-fish contact on horizontal transmission of koi herpesvirus
7. 2019-163 Understanding the genetics and genomics of carp strains and susceptibility to CyHV-3
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What are the carp virus biocontrol risks and how can they be managed?

9. 2017-055 and 2017-056: Water-quality risk assessment of carp biocontrol for Australian waterways
10. 2016-183: Cyprinid herpesvirus 3 and its relevance to humans
11. 2017-127: Defining best practice for viral susceptibility testing of non-target species to Cyprinid herpesvirus 3
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Technical Paper 2. Epidemiology and release strategies

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1.0. About this paper

The National Carp Control Plan (NCCP) has assessed the feasibility of using a virus called Cyprinid herpesvirus 3 (CyHV-3) as a biocontrol agent for invasive European Carp, or common carp (*Cyprinus carpio*, hereafter 'carp') in Australia. This paper summarises and contextualises knowledge emerging from epidemiological modelling under the NCCP. By clarifying how CyHV-3 is likely to transmit among, and impact upon, Australian carp populations, this knowledge will help to determine whether CyHV-3 could be a safe and effective carp control option for Australia. If Australian governments choose to proceed with additional activities to inform a decision on whether or not carp biocontrol should proceed, the research described here would also contribute to development of virus release strategies that maximise effectiveness against carp populations and manage risks.

2.0 Defining epidemiology

Assessing CyHV-3's feasibility as a carp control tool in Australia relies upon the veterinary/medical discipline called epidemiology. The practitioners of this discipline, epidemiologists, study the mechanisms by which disease transmits through, and affects, populations. The *British Medical Journal* defines epidemiology as *'…the study of how often diseases occur in different groups of people and why*' (note that in this definition, 'animals' could be substituted for 'people'). A defining feature of epidemiology is its focus on understanding disease and its impacts at the population level. However, because disease outbreaks are made up of infections occurring in individuals, epidemiologists must also use knowledge from other branches of medicine or veterinary science that study levels of organisation finer than the population (for example, in cells, tissues, and organs).

Most epidemiologists work on disease prevention and control, in either animal or human populations. However, biological control programs in which the control agent is a pathogen (disease-causing organism) or parasite also use epidemiological knowledge to maximise control effectiveness (McColl et al., 2014). Regardless of whether a program aims to prevent disease in valued populations, or to use it as a pest control or eradication tool, there are four broad approaches to epidemiological research (Thrusfield, 2007). A particular study may follow one of these broad approaches but, more often, elements of some or all are employed over the course of an investigation. These general approaches can be classified (from Thrusfield, 2007) as:

- *Descriptive (or observational) epidemiology*, which involves observing disease occurrence and recording possible causative factors. A descriptive approach is often used in the initial stages of an epidemiological study to identify potentially useful pathways for further investigation.
- *Analytical epidemiology*, which uses statistical and diagnostic procedures to interpret and explain observations.
- *Experimental epidemiology*, which involves the controlled application of various factors to groups of animals such that the effects of those factors on disease transmission, infection, and progression can be distinguished from other, complicating (called 'confounding') variables and assessed. Occasionally, features of the study population may allow 'natural experiments'. For example, isolated cattle populations in the Channel Islands helped epidemiologists determine that the causative agent of bovine spongiform encephalopathy ('mad cow disease') was entering herds through contaminated feed rather than through animal-to-animal contact.
- *Theoretical epidemiology*, in which mathematical models are used to represent, explore, and manipulate the behaviour of environments, the populations that live within them, and the diseases occurring within these contexts.

3.0 Epidemiology in the National Carp Control Plan

3.1 Why use a modelling approach?

The NCCP commissioned an epidemiological modelling project (Durr et al., 2019) to improve understanding of likely CyHV-3 dynamics in Australian ecosystems and carp populations. Because it employs a modelling approach, this study fits into the broad category of theoretical epidemiology. However, the modelling process uses data and information collected using analytical, descriptive, and experimental approaches. A modelling approach was selected for the NCCP's epidemiological research because it enabled exploration of virus behaviour in complex, interconnected carp sub-populations, and in river catchments displaying differing flow and temperature regimes. Designing experiments that could encompass the variation through space and time inherent in these complex systems would be costly, time-consuming, and technically difficult. Additionally, the virus must remain in biosecure laboratories until all legislative approvals necessary for its release into the Australian environment have been obtained. This restriction limits the potential for experimental research investigating virus behaviour under conditions similar to those in natural ecosystems (although some aspects of viral biology can still usefully be investigated in controlled laboratories). In contrast, modelling enables manipulation of various parameters within the modelled system to understand their

effects on outcomes. For example, the effects of different carp population densities, water temperatures, flow regimes, and long-term climate cycles and oscillations (e.g. El Niño vs La Niña years) on the virus's population-level impacts can be modelled. Furthermore, data collected by the NCCP's carp biomass estimation project (Stuart et al., 2019) has been incorporated into the models to improve the realism and usefulness of model predictions.

3.2 Balancing complexity and simplicity in modelling

Natural systems are complex, with many interacting components. Determining the extent to which this complexity needs to be incorporated into a model in order to generate useful predictions is a key decision in the modelling process (Grassly and Fraser, 2008). While simple models can often provide the most useful guides to thinking, oversimplification risks excluding variables that could be important in determining outcomes of interest. Conversely, adding too many variables can increase complexity, making model behaviour difficult to interpret (i.e. the modeller loses the ability to explain why the model has produced particular outputs) (Grassly and Fraser, 2008). In the biological sciences, modelling is often most useful as a guide to thinking rather than as a source of definitive answers (Ögüt, 2001). Expressing interactions between the components of natural systems in mathematical terms forces researchers to think clearly about the nature of these interactions, and thereby highlights areas where understanding is poor and additional data collection may be required. By enabling exploration of diverse scenarios, models can also prompt the development of new hypotheses (testable ideas about the way in which a system works) for investigation (Ögüt, 2001).

3.3 An integrated series of models

To avoid repetitive citations, the following discussion of NCCP epidemiological modelling refers to Durr et al. (2019) except where other references are cited. While referred to throughout the NCCP technical papers as 'epidemiological modelling', this component of the NCCP research program actually consists of a hierarchical series of models incorporating

- data on water temperature, inundation and hydrology (water level and river flow),
- habitat suitability metrics (which determine carp abundance) for both juvenile and adult carp,
- carp demography (i.e. the processes that occur within populations to drive growth or decline), and
- the epidemiology of CyHV-3 in Australian carp populations.

Carp biomass estimates generated by the NCCP biomass project (Stuart et al., 2019) integrate into this hierarchy by providing a cross-validation tool for carp abundance estimates generated by the habitat suitability modelling component. Conceptually, the hydrological, habitat suitability, and demographic models create the 'stage' or 'playing field' upon which CyHV-3 epidemiology plays out. Just as the conditions of a sporting field can influence the outcome of a game played on it, so too do these environmental and demographic factors influence the outcome of introducing the virus into carp populations.

3.4 Explaining data-driven modelling

Epidemiological modelling in the NCCP has adopted a 'data-driven' approach. In data-driven modelling, large volumes of real-world data are searched for underlying connections between system variables (Solomatine et al., 2008; Toh and Platt, 2013; Pfeiffer and Stevens, 2015). Data-driven approaches, often termed 'big data', contrast with more traditional, hypothesis-driven approaches to science, which set out to test pre-defined ideas about how the study system works (Solomatine et al., 2008; Pfeiffer and Stevens, 2015). Data-driven approaches to modelling, in epidemiology and more broadly, have been enabled by increases in both computing power and the prevalence of automated data collection through sensors and other devices connected to the internet (i.e. 'the Internet of Things') (Toh and Platt, 2013). To illustrate the data volumes processed in these studies, an investigation of drugs used to treat angioedema (swelling in deeper skin layers) analysed a sample of 100 million people and 350 million person-years of observation (Toh and Platt, 2013). Because data-driven approaches don't rely on pre-determined ideas about how the study system works, they can be useful when studying complex phenomena that are not amenable to formulation of generalised hypotheses (Pfeiffer and Stevens, 2015). For example, the outcome of interest may have many possible causes (Pfeiffer and Stevens, 2015).

Two examples of data-driven approaches in the NCCP modelling are the delineation of river reaches and identification of carp sub-populations that comprise relatively discrete (i.e. non-mixing) units through time. Disease transmission in complex populations spread across large geographic areas requires understanding connectivity between subpopulations (Parratt et al., 2016). Therefore, modelling needed to simulate the connectivity between river reaches, and the carp populations inhabiting them, in a biologically realistic way. Modellers used a data-driven approach based on river flows to delineate river reaches. Using a data-driven approach in this instance meant that river reaches could be defined by a biologically meaningful attribute rather than being subjectively defined by researchers. Additionally, the code and algorithms used can be applied to any catchment of interest, removing the need to define reaches on a catchment-by-catchment basis. Subsequently, river-flow data and carp movement data were combined using a data-driven approach to identify carp subpopulations. In other words, data on river flow and carp movements were allowed to 'speak for themselves' to indicate where and how carp sub-populations could meet and mingle in the real world. In these two examples, data-driven approaches allow a more robust and realistic representation of the real system than could have been achieved if modellers had imposed pre-determined ideas of how the system should work onto the data.

The ability of data-driven approaches to identify previously unseen patterns has led to predictions that big data will render hypothesis-driven research obsolete (Anderson, 2008). Such claims are, however, hyperbolic (Chiolero, 2013; Pfeiffer and Stevens, 2015). While large datasets can undoubtedly yield new insights, and usually do produce robust conclusions, they are not immune to the problems that can beset observational data in epidemiology (Chiolero, 2013). Observational data are those collected when researchers observe the world as it naturally occurs, rather than under controlled experimental conditions. Big datasets are usually observational in nature.

Confounding is a problem of particular concern to epidemiologists working with observational data, including big data. A confounding variable occurs together with a potential cause of disease, and influences the disease outcome, thereby complicating interpretation of the

relative importance of different disease causes. For example, in an epidemiological study investigating the relationship between alcohol consumption and heart disease, smoking would be an important confounding factor, because smoking and alcohol consumption commonly occur together, and both can cause heart disease. To understand the effects of alcohol on heart disease in isolation, investigators would need to 'control' for the effects of smoking. Confounding can occur in datasets of any size, but some authors have argued that data-driven research, with its flexible analytical approaches and lack of pre-specified hypotheses, may be less likely to detect it unless caution is used (Chiolero, 2013). Fortunately, statistical techniques that overcome confounding and other data integrity issues in data-driven approaches are increasing in sophistication, and provide opportunities to exploit the benefits of a larger sample while avoiding potential pitfalls (Toh and Platt, 2013).

Ultimately, data-driven modelling provides epidemiologists with another tool for understanding disease in populations. Uncritical acceptance of big data in epidemiology is as misguided as its outright rejection (Toh and Platt, 2013). Rather, the challenge is to identify ways in which large datasets can be rigorously assembled and creatively combined to yield new insights into complex systems. In the particular case of the NCCP, data-driven modelling provided the only realistic option for answering applied questions about a system characterised by diverse habitat types, a broadly distributed and highly variable target population, and numerous data and knowledge gaps.

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3.5 How does the modelling capture variation across carp's Australian distribution?

Modelling the entire Australian distribution of carp would be prohibitive in terms of data requirements and computing power. Therefore, the modelling focuses on five representative catchments (see Table 1).

Table 1: Catchments modelled for combined hydrological, carp habitat suitability, demographic, and epidemiological modelling in the NCCP

Catchment	State(s)	Reason for selection
Lachlan River	NSW	Contains a diversity of habitat types, and the Lachlan's carp populations were thoroughly studied between 2007 and 2009.
Moonie River	Qld	A representative catchment for warmer habitats in the northern portion of carp's distribution. Additionally, the Moonie River's native fish populations have been well-studied.
Lower Murray River	SA	Carp populations in the lower Murray have been well-studied, providing useful background information for modelling.
Mid Murray River	Vic-NSW	A representative high-flow river; well-studied for carp.
Glenelg River	Vic	A representative coastal river with a geographically isolated carp population. Additionally, a recent carp movement and population study provided useful background for modelling.

For the catchments listed in Table 1, carp populations, river flows, and climatic conditions have been reconstructed for time intervals of 16–28 years (depending upon data availability in each study catchment) enabling modellers to examine the effects of virus introduction under a range of environmental conditions. Against this backdrop of environmental and demographic variability, different epidemiological scenarios relating to transmission rates and mortality in carp populations can be assessed to obtain an understanding of virus behaviour in the highly variable conditions characteristic of Australian environments and carp populations. Inclusion of this variability is a key feature of the modelling, as insights into CyHV-3 dynamics gained from controlled laboratory experiments will not always be applicable to natural environments (Becker et al., 2018).

The modelling allows exploration of virus impacts on carp populations over 5–10 years following virus release. Total reduction in carp density ('total knockdown') is the primary outcome considered by the modelling. However, the extent to which the virus reduces carp populations below threshold densities at which environmental damage may occur (see Technical Paper 1 for further discussion) is also considered. Because the threshold densities at which damage occurs will vary between different species, species groups, and ecosystems, and even within a given ecosystem through time (see Technical Paper 1), three threshold densities—50, 100, and 150 kg ha^{-1}—were used.

3.6 Applying the modelling

Epidemiological modelling results have reshaped thinking on likely CyHV-3 disease dynamics in Australian carp populations. When the NCCP began, expectations were for major carp mortalities and rapid disease transmission across large geographic extents (i.e. an epidemic). These ideas accorded with international reports of large carp kills, particularly in aquaculture (e.g. Haenen et al., 2004), and with the high mortalities reported by the initial CSIRO laboratory trials (see Technical Paper 1).

Epidemiological modelling, combined with analyses of Japanese and North American experience with the virus, has revealed that self-propagating, widespread, and approximately simultaneous carp mortalities are unlikely in Australian ecosystems (Thresher et al., 2018; Durr et al., 2019). Rather, outbreaks killing large numbers of carp are only likely when a particular set of climatic and carp behavioural factors coincide. These results suggest that CyHV-3 would best be used in a targeted way, focussing on schooling (aggregating) carp during relatively narrow seasonal windows.

High densities of susceptible carp is the first of two primary preconditions for an outbreak of CyHV-3-induced disease. The second precondition, water temperature that permits infections and disease, is discussed later in this paper. High carp densities are important because physical contact between infectious and susceptible carp is the most effective CyHV-3 transmission route (Raj et al., 2011; Tolo et al., 2021; Kirkland and Hick 2022). Infectious individuals are those that have the virus, and are capable of infecting others, while susceptible individuals do not have the virus and are vulnerable to infection. In addition to direct physical contact between infectious and susceptible carp, transmission may also occur when carp contact virus particles either floating in the water column or adhering to sloughed skin cells, mucous, faeces or other materials or organisms (Minamoto et al., 2009; Rakus et al., 2013). Epidemiological modelling in the NCCP assumes that transmission requires physical contact, and this assumption has been supported by laboratory experiments demonstrating that physical contact between carp is a far more effective transmission pathway than is horizontal transmission through the water (Tolo et al., 2021; Kirkland and Hick, 2022).

The NCCP experiment comparing transmission pathways (Kirkland and Hick, 2022) generated relative estimates of effective contact rates (denoted by the Greek letter β, 'Beta'), one of the most fundamental rates in epidemiology. Effective contact rate is an important determinant of a disease outbreak's outcomes, so obtaining the best possible estimate for this rate is important (e.g. Nkamba et al., 2019).

In its most basic form, the effective contact rate is obtained by multiplying the total number of contacts between infectious and susceptible individuals per unit time, regardless of whether they result in infection, by the risk of infection inherent in these encounters. However, accurately estimating Beta for real disease outbreaks under field conditions is usually much more complex than suggested by the simple calculation described above, because the various factors that ultimately determine Beta are highly context-dependent. For example, in the case of CyHV-3 transmission, the risk of infection inherent in any given contact between a susceptible and infected carp will depend on factors including water temperature, the rate at which the infected fish is secreting virus, and the immunological state of both individuals. Therefore, the NCCP transmission experiment (Kirkland and Hick, 2022) usefully demonstrates the greater relative importance of physical contact over water-borne virus as

transmission pathways, but does not provide absolute estimates of Beta that accurately reflect those likely to occur in the field.

Obtaining 'field-relevant' estimates of Beta as inputs to NCCP epidemiological modelling remains desirable, but would require more complex experimental conditions, run over longer time-periods than were possible in the transmission experiments conducted under the NCCP (Kirkland and Hick, 2022). The NCCP has recommended that these experiments be undertaken, if governments choose to proceed with further research and planning activities to inform decision-making on carp biocontrol.

Water temperature is the second major precondition for an outbreak. The water temperature range within which CyHV-3 causes disease in carp (the 'permissive range') 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). When carp were infected with the virus in a laboratory at 22 °C and maintained at that temperature for 24 hours, followed by a temperature reduction to 11 °C over four days, there were no mortalities, no clinical signs of disease, and no production of infectious virus, but the virus remained physically present in the infected carp (Sunarto et al., 2014). Above 30 °C viral replication ceases, and the virus becomes harmless to carp (Boutier et al., 2019). Experience with international outbreaks indicates that there is a narrower temperature range, variously cited as 22–24 °C (Becker et al., 2018; Thresher et al., 2018) and 22–26 °C (Hanson et al., 2016) that is optimal for infection and disease. Thus, an effective CyHV-3 outbreak probably requires carp to be in close physical contact with each other, and for water temperature to be within the permissive range (and ideally within the narrower 'optimal range').

Water temperatures throughout much of carp's Australian distribution tend to rise rapidly through the permissive temperature range in spring or early-mid summer, with the exact timing varying regionally. Rising water temperatures and increasing day lengths during this period are also the environmental cues for carp spawning (Smith and Walker, 2004). In response to these cues, carp migrate towards spawning sites in shallow wetlands, leading to high carp densities at in-stream obstacles like weirs, and in spawning habitats (Smith and Walker, 2004; Stuart and Jones, 2006; Stuart and Conallin, 2018). These spring/summer spawning events provide the carp densities necessary to trigger an outbreak, and tend to occur when temperatures are in the permissive range. Thus, virus release would probably involve actively targeting migrating or spawning carp for infection. As aggregations disperse, the intensity of carp-to-carp contacts necessary to sustain transmission will probably break down, resulting in fade-out of the outbreak. That is, the effective contact rate will reduce below that necessary to sustain the outbreak.

While carp aggregation is well-documented in some parts of Australia, the extent to which it occurs across the full geographic extent of the species' Australian distribution is unclear. Further research on carp aggregating behaviour, population structure, connectivity, and demography would provide knowledge that could increase the effectiveness of virus release. Additionally, research of this nature would also provide a valuable foundation for any other carp control methods that might be employed in future if governments choose not to proceed with biocontrol using CyHV-3.

Likely carp knockdown was modelled for a range of virus transmission scenarios. If assumptions underpinning NCCP epidemiological modelling are correct, planned deployment of CyHV-3 could suppress carp densities to approximately 40–60% of pre-release levels (less in some areas, greater in others) for 5–10 years following release. Key assumptions underpinning this outcome are

- There is no pre-existing heritable resistance to CyHV-3 in Australian carp populations. A pilot study under the NCCP supports, but does not definitely prove, this assumption (Durr et al. 2022), and the NCCP has recommended further research to address uncertainties regarding resistance.
- Transmission ceases completely at water temperatures outside the permissive range (defined in the modelling as 16–28°C).
- A proportion of latently infected carp (see Box 1 text at end of paper) will experience reactivation of their infections under permissive conditions. The model is not sensitive to the frequency with which reactivation occurs, but to achieve the carp reductions described above, it must occur. An experiment under the NCCP tentatively supports this assumption under laboratory conditions. In the experiment, temperatures were manipulated to induce latent infections, which then reactivated when temperatures were gradually raised into the permissive range after approximately two weeks (Sunarto and Durr, 2022). However, this experiment was conducted over a brief period under laboratory conditions, used juvenile carp, and was subject to some potentially confounding influences (particularly temperature fluctuations associated with tank-water exchanges). If the virus were introduced into natural ecosystems as a biocontrol agent, latent infections would likely need to be reactivated in adult carp after longer periods of latency (e.g. after the winter months), and infected carp would need to join aggregations. Therefore, if governments choose to continue with further activities to inform an eventual decision on whether or not carp biocontrol should proceed, the NCCP recommends further research investigating latency and recrudescence in adult carp under natural or semi-natural conditions, and over longer time periods than were possible in the preliminary work (Sunarto and Durr, 2022).
- Approximately 80% of infected carp die (i.e. case fatality rate of 80%).

Scenarios with lower case fatality rates (60%), lower effective contact rates (or contact rates that scale linearly with carp density), or in which infections that have become latent under non-permissive temperatures (see Box 1 text at end of paper) do not subsequently reactivate with onward transmission, result in lower knockdowns, ranging from 20–50% and/or population rebuilding within five years.

These insights into likely CyHV-3 epidemiology in Australian ecosystems indicate how the virus might be used to reduce carp populations while managing risks. The value proposition for biological control often lies at least partly in the self-propagating nature of many biocontrol agents; following release, the agent spreads naturally through the target population, and does its job of reducing numbers with minimal management intervention aside from measures to maintain efficacy (Saunders et al., 2010; Peacock et al., 2021). In aquatic ecosystems, however, a self-propagating biocontrol agent that causes high mortality rates in the target organism could be a double-edged sword. While such an agent would likely be effective at reducing the target organism's abundance, cleaning up the dead carp resulting

from epidemics would be challenging. Because epidemiological modelling for CyHV-3 is indicating that outbreaks will be geographically restricted, and only likely when carp aggregation and permissive water temperatures coincide, there may be opportunities to use the virus in a targeted way to control carp populations in key areas where densities are above thresholds for ecological damage (Technical Paper 1). This relatively controlled outbreak pattern predicted for CyHV-3 in Australian ecosystems does not, however, obviate the need for surveillance and monitoring to detect and respond to any unexpected outbreaks. Such outbreaks remain possible through long-distance movements by latently infected carp, deliberate human transfer of infected carp, or other mechanisms. While unlikely to be epidemiologically significant, outbreaks occurring via these mechanisms have important implications of risk management, hence the need for careful surveillance.

From a strategic perspective, virus deployment would need to target relatively large geographic areas and the interconnected carp sub-populations contained therein for simultaneous virus release. This 'broadscale' approach is necessary to (i) prevent restocking of carp sub-populations reduced by viral disease from connected, un-infected sub-populations, and (ii) maximise impacts to carp populations before the potential emergence of herd immunity (see Becker et al., 2018).

Yet, despite the desirability of a broadscale approach, virus deployment across carp's entire Australian distribution within one or two seasons is neither logistically nor biologically feasible. In particular, the 'windows of opportunity' when the water temperatures and carp behaviours conducive to CyHV-3-induced mortalities coincide are relatively narrow, and vary across the species' range according to latitude, elevation, and finer-scale factors such as substrate type and water depth and flow. Given these considerations, virus deployment strategies need to use information from carp biomass and population mapping to identify discrete carp populations, each comprised of sub-populations that connect with each other to varying extents under differing flow regimes, to form the primary 'units' targeted for virus release.

Within these primary virus-release units, achieving maximum carp suppression in the medium- to long-term would be contingent upon (a) deploying virus into as many of the sub-populations comprising a given management unit as possible, and (b) within each subpopulation, ensuring that a sufficient proportion of adult carp are infected to maximise both the initial kill and potential for some carp to survive as latently infected carriers to propagate the virus in subsequent seasons.

Two main virus release methods or techniques have been identified as potentially feasible. First, carp could be captured and inoculated as they begin to aggregate to spawn in spring, before being released back into the waterway in which they were caught. An alternative strategy, dubbed 'Trojan carp' may also have merit, in which carp are caught and inoculated in late winter prior to the onset of aggregating behaviour to initiate a latent infection, and released. During cold weather, carp in some locations may display behaviours that facilitate capture, potentially enhancing the viability of this approach (e.g. Lechelt and Bajer, 2016). As spring approaches and water temperatures increase, these latently infected carp could join spawning aggregations, and should experience temperature-induced reactivation of their infections, thereby infecting other carp and initiating an outbreak. As noted above, an NCCP

laboratory experiment investigating latency and recrudescence (Sunarto and Durr, 2022) provides an initial indication that this release strategy could work, but further work is recommended.

Epidemiological modelling conducted under the NCCP indicates that biocontrol using the carp virus could reduce highly resilient carp populations by approximately 40–60% and less resilient populations by approximately 60–80%. These projected reductions are generalisations and both greater and lesser reductions are expected to occur across the numerous carp sub-populations that constitute Australia's total carp biomass. In some areas where carp biomass is high, even a substantial 40–60% carp reductions may still leave higher densities than would occur in less resilient populations. While any carp reduction has the potential to deliver ecological benefits, these might be optimised through an intensive, targeted 'fish-down' of carp prior to biocontrol operations in high density areas to reduce carp 'starting density'. Assessing the timing, magnitude, and operational planning aspects of this 'pre-fishing effort is beyond the NCCP's scope, but could usefully be investigated by some limited additional modelling

4.0 Can the virus suppress carp populations?

Some scientists have argued that CyHV-3 is unlikely to drive long-term carp population declines (Becker et al., 2018; Marshall et al., 2018; Thresher et al., 2018; Boutier et al., 2019; Kopf et al., 2019; Mintram et al., 2020). Thresher et al. (2018) reviewed carp abundance before and after CyHV-3 outbreaks in North America, and found that carp had only undergone a sustained decline in one of the five study locations. Kopf et al. (2019) argue that the genetic diversity and patchy distribution of wild carp populations could, in combination with the variable physico-chemical regimes characteristic of natural ecosystems, limit mortality from the virus. Marshall et al. (2018) and Kopf et al. (2019) also note that CyHV-3 may have co-evolved with carp over long periods, and that some Australian carp could consequently possess, or rapidly acquire, resistance to the virus. Similarly, Mintram et al. (2020) argued that the emergence of genetic resistance, combined with the high fecundity for which carp are noted, will promote rapid population rebuilding following an initial knockdown caused by viral disease.

Water temperature's central role in CyHV-3 disease dynamics has also led to questions about the virus's effectiveness as a biocontrol agent in the context of the variable water temperature regimes characteristic of many Australian freshwaters. Becker et al. (2018) note that numerous carp will likely be infected by the virus as water temperatures pass through the margins of the permissive range (i.e. as temperatures rise through the upper limits of the permissive range in late spring/early summer, or fall back through its lower limits in autumn). Becker et al. (2018) posit that some of these individuals will survive, developing latent or subclinical infections, and partial, temperature-induced immunity. These individuals could, in turn, expose susceptible carp to the virus at temperatures sub-optimal for disease, leading to the rapid development of herd immunity (Becker et al. 2018). Herd immunity occurs when susceptible individuals are protected from infection by the high proportion of immune, non-infectious individuals around them (Fine, 1993). To develop their arguments, Becker et al. (2018) draw on water temperature data collected below four large dams in the Murray-Darling Basin, observations of CyHV-3-induced disease and viral prevalence in Lake Biwa, Japan, and the Australian experience with another fish virus, the iridovirus called Epizootic haematopoietic necrosis virus (EHNV).

The development of immunity resulting from infections occurring outside, or on the margins of, the permissive range differs from the development of true genetic resistance driven by natural selection (Becker et al., 2018). Selection for resistance to a disease occurs because, within any population of organisms, some individuals will likely possess genes that make them either resistant to infection by a given pathogen, or capable of defeating disease following initial infection (Lipsitch and Sousa, 2002; Karlsson et al., 2014). When a population encounters a new pathogen for the first time, these resistant individuals remain unaffected, or only develop non-serious disease, while the broader population to which they belong experiences high mortality or debilitating disease that reduces reproductive success (Karlsson et al., 2014). By virtue of their increased survivorship and/or fitness, resistant individuals have relatively greater reproductive success, so genes that code for resistance tend to spread through populations (Lipsitch and Sousa, 2002; Karlsson et al., 2014). Eventually, most of the population becomes resistant to the disease (Karlsson et al., 2014).

While the basic concept of natural selection favouring resistant individuals is relatively simple, the manner in which selection by infectious diseases plays out in real populations is usually quite complex. This complexity arises because not only does the pathogen apply selective pressure to the host (i.e. by killing non-resistant individuals, or reducing their reproductive success), but the pathogen also changes (evolves) in response to selective pressures, particularly those related to optimising transmission (Di Giallonardo and Holmes, 2015). Adding to this complexity, the time taken for resistance to develop through natural selection depends upon factors including

- the proportions of resistant and non-resistant individuals in the population,
- the relative balance of evolutionary costs and benefits inherent in resistance (e.g. the genes that confer resistance may also code for a trait(s) that has negative fitness consequences),
- the extent to which the resistance genes confer protection against disease (i.e. whether protection is complete or partial) (Lipsitch and Sousa, 2002), and
- potentially, the extent to which resistance depends on interactions between multiple genes, a phenomenon called epistasis (Hall and Ebert, 2013).

Predicting how long genetic resistance will take to emerge for a given host-pathogen system is therefore complex.

A preliminary study indicates that the gene variants (alleles) thought to confer resistance to CyHV-3 are not present in Australian carp populations, implying that the evolution of true genetic resistance could take well over a decade (Durr et al., 2022). However, this work did not constitute a comprehensive genetic survey of Australian carp populations, and was exploratory in nature (and intended as such). Indeed, the genetic basis for resistance to CyHV-3 remains imperfectly understood, but is subject to ongoing investigation by international researchers interested in breeding resistant carp strains for aquaculture (Durr et al., 2022). The primary value of the NCCP research investigating resistance was in developing appropriate tools to further study the emergence of resistance in Australian carp (Durr et al., 2022). The NCCP has recommended additional investigation of resistance, if governments

choose to continue with activities to inform an eventual decision on whether or not carp biocontrol should proceed.

Apart from the role ascribed to water temperature as a driver of herd immunity in the framework of Becker et al. (2018), there are likely to be many Australian freshwater habitats in which temperature rises rapidly through the permissive range in spring and early summer, and remains too high for reliable infection for extended periods. Temperature regimes like this will occur in Qld and northwestern NSW, and in shallow wetlands throughout carp's Australian distribution. High temperatures also create the possibility of 'behavioural fever', wherein infected carp actively seek out warmer water to deactivate the virus (Rakus et al., 2017). At the opposite end of the temperature spectrum, coldwater pollution (release of cold water from the deep layers of dams, properly termed 'hypolimnetic release') could result in extended river reaches where temperatures remain below the permissible level for CyHV-3 (Kopf et al., 2019). Coldwater pollution is both more spatially restricted (i.e. to the river reaches below dams) and amenable to management than are high temperatures, but will nonetheless need to be considered when developing CyHV-3 release strategies (Kopf et al., 2019).

5.0 Conclusions

Safe and effective carp biocontrol requires understanding CyHV-3's likely behaviour in, and impacts on, Australian carp populations, then using this knowledge to develop virus release strategies that maximise effectiveness and manage risks. These goals are essentially challenges in applied epidemiology. Epidemiological research almost always requires knowledge of the study population's abundance, density, and distribution through space and time, so carp biomass estimates (Stuart et al., 2019) are important to this, and many other, facets of the NCCP research program.

The epidemiology of infectious diseases is always contingent upon host demography and behaviour, and upon the physical and climatic features of the environments in which disease plays out. Therefore, a key feature of epidemiological modelling in the NCCP has been developing sound environmental and demographic contexts within which disease dynamics can be explored. In particular, reconstruction of carp populations over extended time periods (ranging from 16–28 years, depending on study catchment) provides a powerful tool for assessing the likely magnitude of carp reductions in the context of their typical 'boom and bust' abundance cycles.

Uncertainties regarding CyHV-3 epidemiology in Australian environments remain. While genetic (heritable) resistance to CyHV-3 may evolve slowly in Australian carp populations, this has yet not yet been unequivocally demonstrated. Further targeted research investigating the emergence of resistance in Australian carp could reduce this uncertainty. The rapid emergence of herd immunity as a result of infections around the margins of the virus's permissive temperature range is also possible. Likewise, some regions and habitat types within carp's Australian distribution are likely to experience temperatures within the permissive range for shorter time periods than others. Examples include dryland rivers in the northern Murray-Darling Basin, and areas subject to coldwater pollution. The extent to which these factors could influence broadscale effectiveness of the virus as a biocontrol agent is unclear. If governments choose to proceed with further activities to inform decision making

on carp biocontrol, response measures such as targeted use of physical removal in these areas could be considered to bolster biocontrol effectiveness.

Modelling, combined with insights from the broader scientific literature, indicates that CyHV-3 is only likely to cause major outbreaks when water temperatures in the permissive range combine with carp aggregation events. This concurrence of events is most likely when carp are aggregating to spawn in spring and early summer. Carp aggregation events are important in triggering outbreaks because direct physical contact between carp is very likely to be the most effective virus transmission pathway. The picture of CyHV-3 disease dynamics that has emerged from the epidemiological modelling described here indicates that use of the virus as a biocontrol agent will require a highly targeted virus release strategy, involving identification of aggregation locations and subsequent targeted infection.

Biological control is often favoured as a management approach because biocontrol agents tend to be self-propagating within pest populations, and therefore require relatively little management intervention beyond monitoring and ongoing measures to maintain efficacy measures (Saunders et al., 2010; Peacock et al., 2021). In contrast, the epidemiological modelling described here indicates that biocontrol using CyHV-3 will require a more proactive deployment approach, in which interconnected carp sub-populations are identified and individually targeted during an active operational phase. Within this framework, release strategies are required that ensure infectious carp enter spawning aggregations, where high rates of physical contact should ensure they infect numerous susceptible carp.

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Box 1 text: Viral latency explained

Latency and subclinical infection are virologically distinct but, in the particular context of carp biocontrol, have similar epidemiological implications. In virology, 'latency' refers to a strategy used by some viruses, including herpesviruses, to 'hide' 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 cell division, but do not replicate by 'hijacking' the host cells (see Technical Paper 1 for an explanation of viral replication). 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, and likely alloherpesviruses, 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 the alloherpesvirus 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 infections can undoubtedly follow a trajectory that is highly indicative of latent and/or subclincal infection (Sunarto et al., 2014; Boutier et al., 2015). Diseased carp can 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 alloherpesviruses, potentially indicating chronic productive infection rather than true latency (Sunarto et al., 2014). Conversely, there is evidence that carp white blood cells could be the location where latent virus 'hides' from the host immune system (Michel et al., 2010; Eide et al., 2011; Xu et al., 2013; Reed et al., 2014). Regardless of whether CyHV-3 exhibits true latency or chronic productive infection, carp in this phase of infection do not appear to produce infectious virus (Sunarto et al., 2014).

NATIONAL CARP CONTROL PLAN

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