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Investigating Cyprinid herpesvirus 3 as a potential biocontrol agent for carp

International study tour

Matt Barwick and Dean Gilligan

NSW GOVERNMENT Department of Primary Industries

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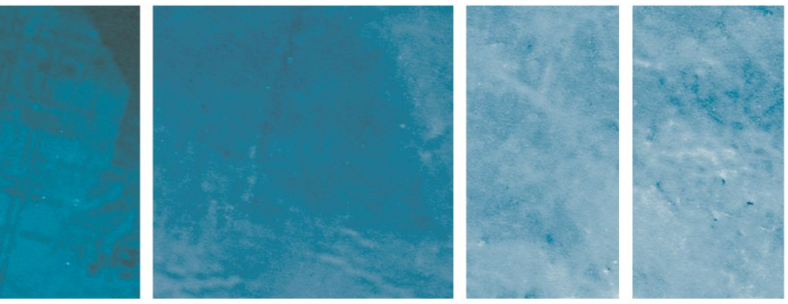

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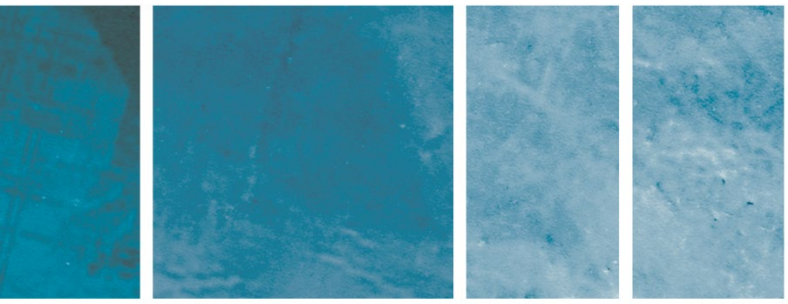


The international study tour was undertaken by officers of NSW Department of Primary Industries in June/July 2015.

This study tour was completed as a component of research funded under the Invasive Animals Cooperative Research Centre (CRC), which ended on 30 June 2017 and has now become Centre for Invasive Species Solutions (CISS). Also note the Invasive Animals CRC website will be inactive after 30 June 2018.

Abbreviations

AAHL – Australian Animal Health Laboratories

CEFAS - Centre for Environment, Fisheries and Aquaculture Science

CRC – Cooperative Research Centre

CSIRO Commonwealth Science and Industrial Research Organisation

CyHV-3 - Cyprinid herpesvirus 3

DNA - deoxyribonucleic acid

FRDC – Fisheries Research and Development Corporation

NCCP – National Carp Control Plan

OATA - Ornamental Aquatic Trade Association

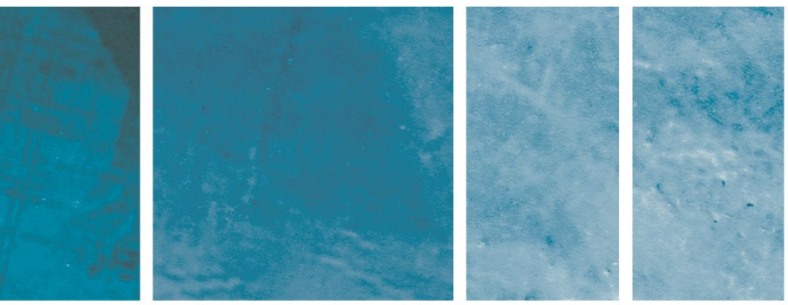
Summary

Common carp (*Cyprinus carpio*) are among the eight most invasive fish species in the world, make up a high percentage of fish biomass in many Australian waterways, and are now found in all states and territories with the exception of the Northern Territory. Eight years of research conducted by CSIRO with funding through the Invasive Animals Cooperative Research Centre (CRC) has shown a naturally occurring virus now occurring within 33 countries known as cyprinid herpesvirus 3 (hereafter referred to as the carp herpesvirus or CyHV-3) to offer potential as a biological control agent for carp in Australia. This research has demonstrated that under the right conditions carp present in Australia are susceptible to CyHV-3, and that the virus will not cause disease in non-target species.

The New South Wales (NSW) Department of Primary Industries delivered a parallel project also funded under the Invasive Animals CRC to develop a biological control strategy for carp, progress requisite government approvals, and establish scientifically robust ecological benchmarks to enable assessment of recovery of freshwater habitats in response to carp biocontrol. An international study tour was undertaken in June 2015 to address key knowledge gaps associated with the potential use of the carp herpesvirus for the biological control of carp in Australia. Objectives of the study tour were to:

1. advance development of a biosecurity plan for koi producers
2. Inform development of an effective and cost-efficient post-release clean-up strategy
   a. Collect information on long-term effects of outbreaks on wild carp populations
   b. Determine rate of decline in carp immune response
3. Confirm progress and timeframes for development of an inactive vaccine for the carp herpesvirus
4. Gather any information to assist in evaluating human health implications

A program of meetings was arranged with researchers, fisheries managers and industry representatives in Indonesia, the United States, United Kingdom, Israel and Japan with knowledge of CyHV-3, its effectiveness on the target species and strategies for preventing undesirable impacts. Key outcomes delivered through the study tour, and activities undertaken to pursue each outcome are discussed in this document, and recommendations are summarised below.



Recommendations

- Several successful biosecurity strategies were identified with significant communality in approach. It is recommended that information collated be used as a resource for future work to co-design a biosecurity strategy tailored for Australian context in collaboration with representatives of Australia's Koi community.
- There was limited success sourcing datasets to enable consideration of possible long-term impacts of virus outbreaks. it is recommended that a more extensive review of unpublished data from Japan and/or North America be undertaken. This work should look for any datasets of carp abundance pre/post outbreaks, compare and contrast any impacts observed, and consider variables that might contribute to reported outcomes.
- Clean up methods employed were generally labour-intensive and lacking in systemisation. This was largely due to the unexpected nature of fish kill events, and a need for rapid response, often with limited resources. On the basis of information collated it is recommended that there is a need for a broader review of methods used to undertake fish kill clean up activities, and not limit this work only to those caused by CyHV-3 in carp. This work should consider methods used in specific habitat types, resources required, issues encountered, and levels of effectiveness.
- Progess with development of an inactive vaccine is slow, though ongoing. It is recommended to maintain a watching brief over progress with developing an inactive vaccine, to enable consideration of implications for use in an Australian context.
- Whilst risk of infection for humans is also considered to be extremely low/negligible, it is also recognised that this may be an area of concern for the Australian public. It is recommended that indepent expertise in zoonoses be engaged to inform consideration of relevant risks to human health, and inform consideration of future work required.

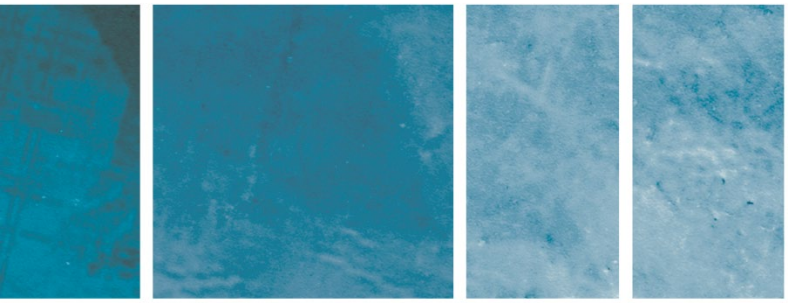
Introduction

Common carp (*Cyprinus carpio*) are now found in all Australian states and territories with the exception of the Northern Territory, and make up a significant proportion of fish biomass in many Australian waterways. In 2002, CSIRO's Australian Animal Health Laboratories (AAHL) began investigating the potential use of a naturally occurring virus known as cyprinid herpesvirus 3 (CyHV-3, also referred to as the carp herpesvirus) as a biocontrol agent for carp in Australia. This involved testing non-target species for infectivity, including several species of native Australian fishes, rainbow trout, and model species of reptiles, crustaceans, amphibians, birds and mammals; establishing that Australian carp populations are susceptible to disease; developing a culture and detection methodology; and screening the Murray–Darling Basin population for any herpes-like viruses that may confer resistance. Results obtained suggest that the carp herpesvirus represents a potentially viable and effective biological control agent for carp in Australia. Use of a viral biocontrol agent for carp in Australia may require approvals under several pieces of legislation including the *Quarantine Act 1908*, *Environmental Protection and Biodiversity Conservation Act 1999*, the *Agricultural and Veterinary Chemicals Code Act 1994*, and the *Biological Control Act 1984*. The New South Wales (NSW) Department of Primary Industries is leading a project funded through the Invasive Animals Cooperative Research Centre (CRC) to (among other things) progress necessary government approvals under relevant legislation. It was recognised that a significant body of knowledge on the virus and its behaviour, impacts on carp, and ecosystem implications exist in unpublished form, and would contribute data to inform legislative approval processes. The need was identified to engage with researchers, managers, industry representatives and interest groups in countries with long histories of dealing with this virus, to seek access to compile information to inform development of Australia's carp biocontrol plan.

Four primary objectives were pursued through the study tour.

1. Advance development of a biosecurity plan for koi producers

Koi carp are an ornamental strain of *Cyprinus carpio*, and so are also vulnerable to the carp herpesvirus. Koi carp production and ownership is legal in New South Wales, Western Australia and the Australian Capital Territory (ACT), and there is concern among producers and enthusiasts that their livelihood/hobby may be directly threatened by release of the carp herpesvirus. In contrast to this presumption, successful koi production persists in many countries where the carp herpesvirus is known to occur including Israel, Japan, the United Kingdom and the United States. In these and other countries, the presence of the carp herpesvirus within waterways on koi breeders and hobbyists has been mitigated through availability of a live attenuated vaccine for the virus and use of effective biosecurity protocols. Meetings took place with fish health specialists, virus vaccine developers and koi producers in Israel, Japan and the United Kingdom to develop a detailed understanding of biosecurity measures employed. Information gathered was intended to assist in future development of a national carp herpesvirus biosecurity plan.



2. Inform development of an effective and cost-efficient post-release clean-up strategy

Under appropriate conditions the carp herpesvirus can cause high mortality levels in carp. Carp are understood to constitute a significant proportion of fish biomass in Australian rivers. Consequently there is a pressing need to develop a highly efficient clean-up strategy able to be implemented to manage risk to human health, water supply security, human amenity and ecosystem function in response to significant carp kill events. Only a small number of large-scale outbreaks have been documented within temperate regions for which data may be available on clean-up methods used, associated costs, effectiveness and human health implications. Meetings took place with waterbody and fishery managers at key locations to expand the project team's network of expert contacts in this field, and seek access to unpublished information which will inform development of a national carp clean-up strategy for Australia.

2a. Collect information on long-term effects of outbreaks on wild carp populations

Though high carp mortality rates have been reported in association with CyHV-3 outbreaks overseas, vary few case studies exist that describe long-term impacts of the virus on carp populations in published literature. Consequently, the need was identified to consult with fisheries managers and researchers in countries with a long history of interaction with the carp herpesvirus to identify any unpublished datasets available for consideration.

2b. Determine rate of decline in carp immune response

A key concern held by some relating to the use of the carp herpesvirus for the biological control of carp is that surviving fish may quickly develop immunity, enabling a population of resistant carp to re-establish over time. Sourcing of long-term datasets would enable the likely rate of decline in immunity to be determined, and help inform consideration of a suitable frequency for re-application of the virus. There are only a small number of locations for which long-term data (more than 10 years) might be available to enable investigation of immune response in carp including Lake Chautaqua in the United States, Lake Biwa in Japan and Cirrata Reservoir in Indonesia. Data, if available, is unpublished and so not publicly available. Meetings were scheduled with researchers who have studied CyHV-3 outbreaks in these and other candidate locations to negotiate access to, and use of, relevant datasets.

3. Confirm progress and timeframes for development of an inactive vaccine for the carp herpesvirus

Several laboratories around the world are using a range of strategies to develop vaccines for the carp herpesvirus. A live attenuated vaccine has been developed and used in Israel to control the carp herpesvirus since 2004, and was later approved for importation and use in Indonesia and the United States, with plans to make the vaccine available more broadly.

There are no plans to make a vaccine for the carp herpesvirus available for use in Australia at this time. This is because the availability of a live attenuated vaccine in Australia would present an unacceptable risk that vaccinated fish might escape into the wild, and result in establishment of a large population of carp that were resistant to the biocontrol agent in the wild.

It is understood that an inactivated liposome-based oral vaccine candidate with 70 per cent efficacy was described in Japan, and research into DNA vaccines against CyHV-3 are also reported, with a United States patent submitted in 2011 for this method of immunisation.

Meetings were held with relevant experts to clarify risks posed by vaccines to outcomes for Australia's carp biocontrol program and develop an understanding of progress made with respect to development and production of inactivated and DNA vaccines, to consider their possible role in the protection of ornamental koi in Australia.

4. Gather any information to assist in evaluating human health implications

Proposed use of the carp herpesvirus as a biological control agent for common carp would initially result in high levels of human interaction with the virus, both through contact with contaminated water and infected fish through handling.

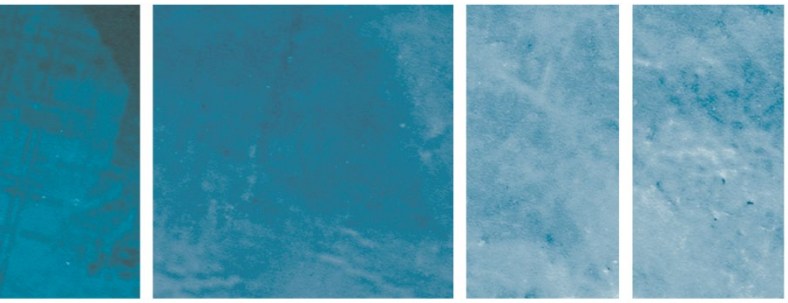
Human health implications of the carp herpesvirus are not explored within the published literature, despite decades of human interaction with the virus. Consequently, there was a need to gather any information available on human health issues that had been documented in response to exposure to the virus, or infected fish.

Results and discussion

NSW Department of Primary Industries officers participated in an international study tour during June/July 2015 as a deliverable of Invasive Animals CRC project 3.W.2.
A schedule of meetings were undertaken with researchers, managers, industry representatives and interest groups in the United States, the United Kingdom, Israel and Japan to address the previously described objectives (see Appendix 1 for the itinerary). Key outcomes delivered are summarised below.

1. Advance development of a national biosecurity strategy

Interviews were conducted with professional koi producers in the United Kingdom (Hatchery Manager at Neil Hardy Aquatica, reputedly the largest English ornamental fish retailer); Israel (Manager of Dag Noy Kfar Ruppin; Manager of Hazor'ea Aquatics, understood to be fourth largest fish producer in the world and second biggest importer into Europe; Research Manager with Ma'agan Michael); and Japan (Manager of Nishikigoi Village Oija; one of the larger Nishikigoi breeders in Japan's Niigata District; and a well-known veterinarian and award winning Nishikigoi owner. A number of industry representatives were also interviewed including United Kingdom manager of the Ornamental Aquatic Trade Association (OATA) and Director of the International Nishikigoi Promotion Center in Japan. Key researchers from the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) and Head of Niigata Prefectural Freshwater Fisheries Experimental Station were also interviewed. These interviews enabled strategic



biosecurity plans to be considered, methods to be collated and effectiveness levels examined

Three main strategies were employed by breeders and enthusiasts to mitigate impacts of the carp herpesvirus. Some relied on use of a live attenuated vaccine developed in Israel in 2004 (KV3) to protect fish, others utilised biosecurity practices and some employed a mix of both strategies.

Biosecurity plans developed and maintained by CEFAS, OATA, the Lincolnshire Fish Health Laboratories and Research Centre, and the Niigata Prefectural Freshwater Fisheries Experimental Station were also sourced to assist in future development of a national biosecurity strategy. Clear parallels were identified within biosecurity protocols adopted in the United Kingdom, Israel and Japan that were reported to be vital for any successful biosecurity strategy. In particular, quarantining of new fish for three to four weeks prior to introduction into a resident population was reported to be critical, as was controlling traffic into/out of fish containment areas and between ponds/tanks. Sterilisation of equipment and disinfection of hands and feet when moving in/out of a facility or between sections were also commonly employed, and many also maintained separate food supplies, equipment and water sources for each tank/pond to enable containment in the event of an outbreak.

Large-scale breeders Neil Hardy Aquatica in the United Kingdom and Hazor'ea Aquatics in Israel were observed to rely entirely on application of biosecurity protocols to prevent carp herpesvirus incursions, with high reported success. Israeli aquaculture facility Ma'agan Michael produce both vaccinated koi, and biosecure koi within two separate sections of the one facility. The ability to do so in close spatial proximity was indication of the effectiveness of biocontrol measures in use.

Discussions with representatives of CEFAS in the United Kingdom highlighted key differences in historical records of CyHV-3 observations within recreational fisheries and commercial fish farms that offer insights into variables that elevate risk of infection/outbreak. At the time of writing there had been approximately 100 outbreaks recorded in the United Kingdom since the disease caused by the CyHV-3 was declared notifiable. All of these reported outbreaks occurred within recreational fisheries. None were recorded from ornamental fish breeding facilities. It is noteworthy that a key difference in the management of fisheries and fish farms is that all farms are required to have a biosecurity plan in place, whereas there is no such requirement for recreational fisheries. This observation further supports the effectiveness of biosecurity controls in protection against virus incursions.

A general trend of increasing frequency of CyHV-3 outbreaks since 2007 in the United Kingdom is notable (see Figure 1). It is thought that this is in response to favourable environmental conditions (high temperatures, low dissolved oxygen levels), and an increasing trend towards high stocking rates of recreational fisheries. The impact of these factors should be considered in development of any virus release strategy for Australian waterways.

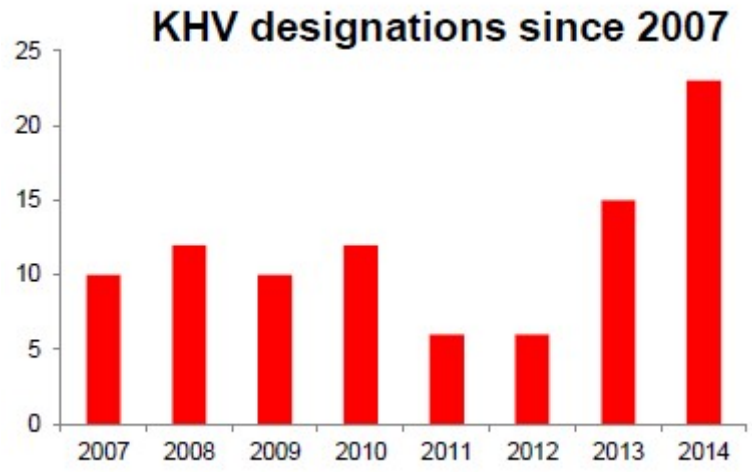


Figure 1. Reported incidences of CyHV-3 (KHV) 2007–14. None were reported in the fish farming network at time of writing - all were in recreational fisheries

There is noted to be considerable expertise within the Koi community and associated industry on this issue, including fish health professionals who service the industry. Further, there is potential for unique attributes and practices within Australia's commercial and recreational Koi operations that are not reflected elsewhere. Consequently, it is recommended that information gathered during this study tour be used as a resource when engaging an appropriately skilled fish health specialist to co-design a biosecurity strategy in partnership with representatives of Australia's Koi community, that is applicable to an Australian context.

2. Inform development of an effective and cost-efficient clean-up strategy

Study tour participants met with industry bodies, community groups, and research organisations in the United States and Japan to learn about clean-up methods employed following significant carp kill events. In particular, attempts were made to source information on resourcing, methods used and timeframes involved, levels of effectiveness, safety measures employed and human health issues encountered.

Members of the Chautauqua Lake Association in New York state were interviewed in relation to a significant fish kill in Lake Chautaqua that occurred in 2004 in response to an outbreak of CyHV-3. In two weeks approximately 500 tonnes of carp succumbed to the disease caused by the virus, approximately two weeks before the busiest holiday season, the 4th of July.

Despite the scale of the carp kill, staff from the Chautauqua Lake Association reported ability to address the fish kill in approximately two weeks using seven boats manned by teams of four. Staff used shovels and pitchforks to collect dead carp, depositing them into bins, which were then transferred to utility trays lined with canvas, and then deposited

into limed pits and buried. Dissolved oxygen, turbidity, phosphorus, nitrogen and E.coli bacteria were monitored during the outbreak. Water quality impacts were considered minor and short term in duration. Participants in clean-up efforts indicated they would not have done anything differently in terms of the methods employed. Protective clothing was used initially during clean-up efforts, however many staff were reported to later dispense with the use of protective gloves, suits and boots as they felt them to be cumbersome/unnecessary. Whilst this is not recommended, no human health issues were reported to result from participation in clean-up activities.

Interestingly, representatives of the Chautauqua Lake Association reported that the carp herpesvirus had not caused any subsequent fish kills in the lake since 2004, nor did it cause fish kills in adjacent connected waterways in 2004. This was unexpected given the highly virulent reputation of the carp herpesvirus within the published literature.

Figure 2. Chautauqua Lake Association staff undertaking activities to clean up carp kill, Lake Chautaqua, 2014.

Project members also met with researchers from Japan's Ministry of Agriculture, Forestry and Fisheries, and commercial fishers to learn about methods employed for cleaning up a significant fish kill in Lake Biwa, Japan. Lake Biwa is 670 square kilometres in area. A small carp mortality event caused by CyHV-3 was observed in autumn of 2003, and a significant mortality event occurred the following spring, in which an estimated 100,000 carp died. There have been estimates that the fish kill in spring 2004 represented 70 per cent of carp biomass in the lake1, but this is an estimate only, and may be an overestimate of the proportion of carp lost.

Study tour participants learned that methods used for cleaning up the fish kill closely mirrored those used for Lake Chautaqua in 2004. Small teams of fishers used their boats and nets, working along the downwind side of the lake to collect dead carp, which were then either transported to limed pits for deposition, or incinerated. Interestingly, those interviewed indicated that smaller carp were not affected, however it may have been

[1] http://www.uni-muenster.de/journalisten/pdf/KHV_symposium_2011.pdf

that smaller individuals were more easily broken down or scavenged and so were not reflected in fish kill events observed. The clean-up process was estimated to take 'several weeks' and odour was noted to persist in the local area for approximately one month.

The total cost of clean-up activities was unquantified as much of the work was undertaken by members of the community or commercial fishers who did not recoup costs. No changes in water quality were recorded.

Similarly to the Lake Chautauqua case study, no large-scale fish kills resulting from CyHV-3 outbreaks were subsequently recorded in Lake Biwa, however it is understood that small numbers of mortalities have been reported in subsequent years.

The rate of spread of CyHV-3 throughout Japan was noted to be significant, with studies demonstrating presence of the virus in 90 per cent of rivers in Japan within five years of initial detection in Lake Kasaumagaura in 2003.[2] Those interviewed upheld a suggestion within the published literature, that virus invasion does not consistently cause an outbreak and that several environmental factors may be involved in causing carp kill events.

Unfortunately no records on carp abundance are maintained by Japanese local or federal government, or fishers of carp populations in Lake Biwa, as they do not target this species for human use. Consequently, it was not possible to determine long-term impacts on the resident carp population, however anecdotally commercial fishers interviewed indicated carp numbers had rebounded to some extent within three to four years.

[2] http://www.uni-muenster.de/journalisten/pdf/KHV_symposium_2011.pdf

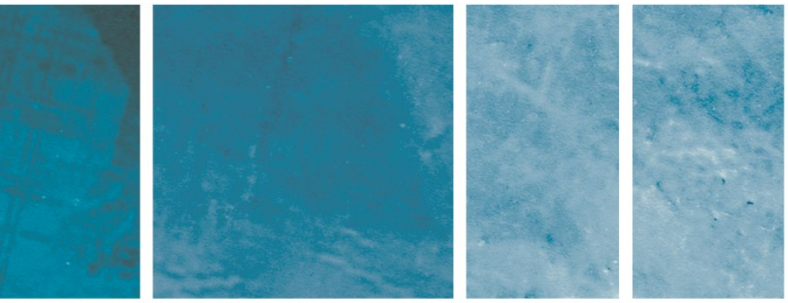


Figure 3. NSW Department of Primary Industries staff interviewing commercial fishers and researchers on the shores of Lake Biwa.

On the basis of information collated it is recommended that there is a need for a broader review of methods used to undertake fish kill clean up activities, and not limit this work only to those caused by CyHV-3 in carp. This work should consider methods used in specific habitat types, resources required, issues encountered, and levels of effectiveness.

2a. Confirm long-term impacts of virus outbreaks

Members of community groups, fisheries management agencies, commercial fishers and researchers were interviewed in the United States, the United Kingdom and Japan to seek access to long-term datasets on carp abundance following outbreaks of the disease caused by CyHV-3.

Representatives of the New York Department of Environment and Conservation were interviewed in relation to the 2004 outbreak in Lake Chautauqua to seek access to any datasets on carp abundance before/after the event that might provide an insight into long-term effects of the carp herpesvirus on populations. Fish survey reports were searched dating back to 1937, however despite routine collection of data on sportsfish species in the lake and elsewhere in the region, unfortunately no datasets on carp abundance were identified.

Anecdotally, conflicting views regarding long-terms impacts of CyHV-3 on carp populations in Lake Chautauqua were reported by representatives of the Chautauqua Lake Association and the New York Department of Environment and Conservation. Chautauqua Lake Association staff expressed the view that carp were no longer present in high numbers in the lake following the outbreak in 2004. By comparison staff of the Department of Environmental and Conservation indicated that the species was once again relatively abundant within the lake by the time of writing.

It was also not possible to ascertain long-term responses in carp abundance after the outbreak in Japan, as common carp are generally not targeted for human use, and so it is understood that no data is collected. Commercial fishers operating in Lake Biwa anecdotally reported recovery in catch rates following the 2004 outbreak which reportedly caused 70 per cent mortality within the wild carp population within three to four years. Unfortunately this could not be substantiated with data.

It was not possible to ascertain long-term impacts following virus outbreaks within UK waterways as they tend to be observed within recreational fisheries which are artificially stocked, thereby masking any long-term effect.

Individuals interviewed in the United States, the United Kingdom and Japan consistently reported a very low incidence of recurring outbreaks, suggesting the likelihood of naturally recurring outbreaks in Australia following release may be low. Given that these observations were made in instances where virus outbreaks were considered to be undesirable, application in an Australian context, where the virus will be actively reintroduced in a manner and timing intended to cultivate repeat outbreaks, should be made with caution.

Due to limited success in identifying datasets to enable consideration of possible long-term impacts of virus outbreaks it is recommended that a more extensive review of unpublished data from Japan and/or North America be undertaken. This work should look for any datasets of carp abundance pre/post outbreaks, compare and contrast any impacts observed, and consider variables that might contribute to reported outcomes.

2b. Determine rate of decline in carp immune response

Researchers from Cornell University's Veterinary Medicine Centre in Ithaca, New York were interviewed to seek access to datasets that might inform assessment of how carp immunity may change over time. Study tour participants were advised that researchers had sampled carp from Lake Chautauqua five years after the 2004 fish kill to determine whether the virus was still prevalent within the lake's carp population. Though sample sizes were low, they found 95 per cent of surveyed fish old enough to have experienced the initial outbreak to be virus-free after five years (see Figure 4).

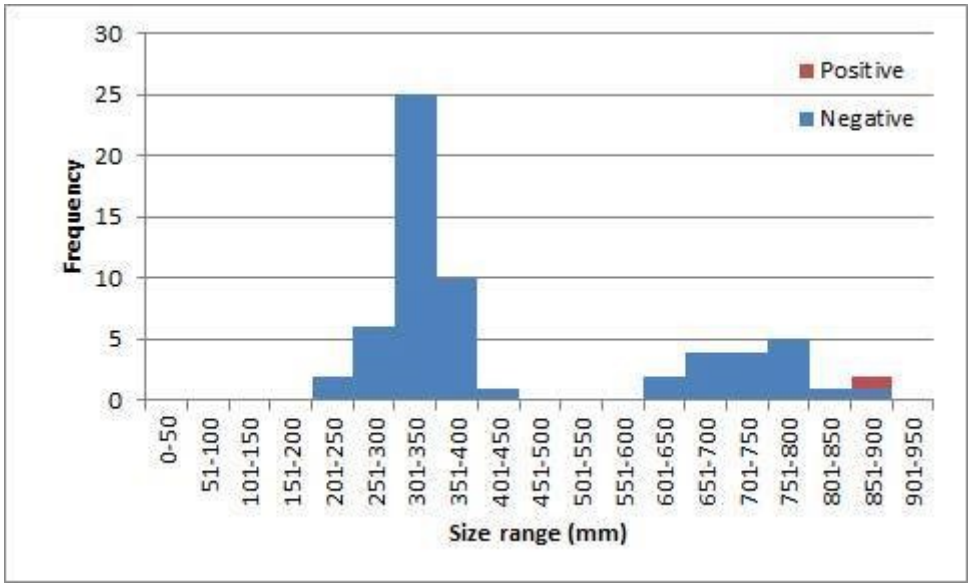
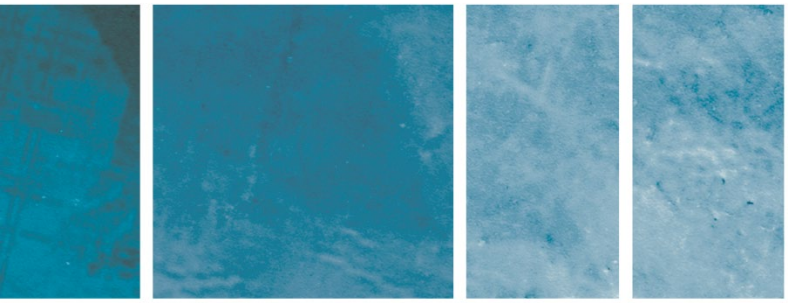


Figure 4 shows that only a small number of carp sampled five years after the initial outbreak of the carp virus in Lake Chautauqua were infected with the carp virus, and all were large (so were either survivors of the initial outbreak, or immigrated from elsewhere (courtesy of Cornell University's Veterinary Medicine Centre).

Researchers also collected blood samples from fish sampled, and will attempt to test for antibodies to determine whether the sampled fish have previously mounted an immune response to CyHV-3. These findings suggest that carp may be vulnerable to reinfection approximately five years after an initial outbreak.

The project team interviewed staff from KoVax Ltd in Israel, who developed a live attenuated vaccine for CyHV-3 (KV3). Data collected by KoVax staff on immunity to the



live attenuated vaccine revealed a significant reduction in antibodies between 3 to 6 months post vaccination (see Figure 5).

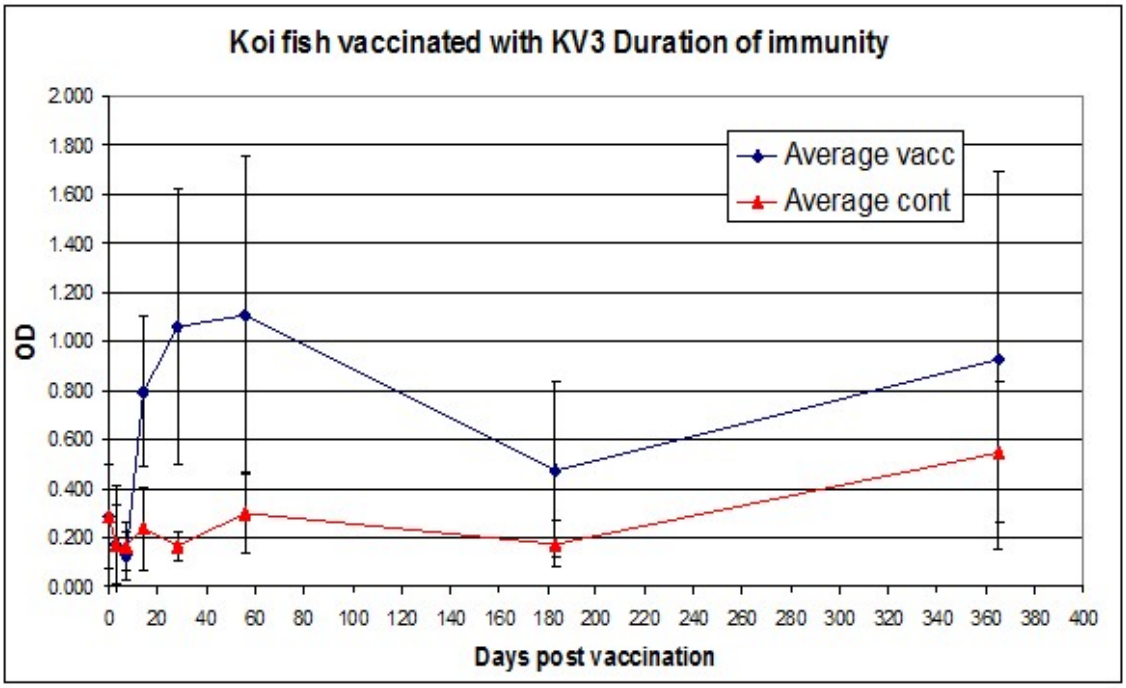


Figure 5. Duration of immunity among fish vaccinated with KV3 (each datapoint represents approximately seven fish).

Additional evidence was obtained from trials undertaken by the research and development manager for Ma'agan Michael, one of the largest producers of carp for human consumption and ornamental purposes in Israel, demonstrating a significant reduction in immunity only 12 months after vaccination, however immunity was able to be increased through administration of a booster vaccine (see Table 1).

Table 1. Survival post challenge with CyHV-3 at 1, 6, 12 and 14 months after vaccination with KV3.

		Survivability
Time of challenge	Test	Control
1 month	90%	13%
6 months	70%	6%
12 months	37%	3%
14 months*	78%	0

* Following booster

These findings suggest a more rapid decrease in survivability after vaccination to those reported by O'Connor et al. (2014), who observed a reduction in survivability from 90 per cent one month after vaccination to 64 per cent 13 months after vaccination with KV3.

3. Confirm progress and timeframes for development of an inactive vaccine for the carp herpesvirus

The Head of Diagnosis group within the Diagnosis and Training Center for Fish Diseases, National Research Institute of Aquaculture, Fisheries Research Agency was interviewed to determine progress in the development of an inactive vaccine. Project team members learned that this project had been discontinued due to the inability to develop an inactive vaccine able to be commercialised. Subsequent discussions with researchers in Israel revealed that work is ongoing to develop an inactivated preparation, however the timelines to availability are uncertain at this time.

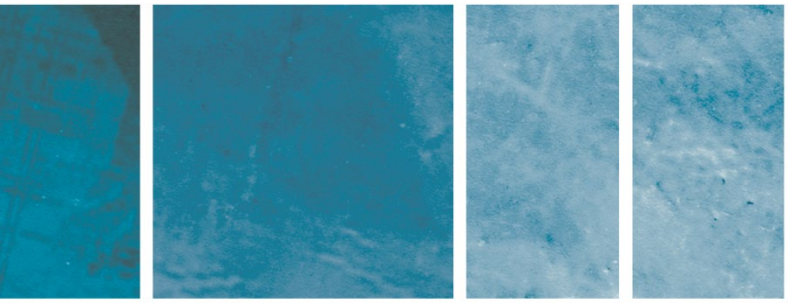
It is recommended to maintain a watching brief over progress with developing an inactive vaccine, to enable consideration of implications for use in an Australian context.

4. Consider human health implications of CyHV-3

The project team met with representatives of Indonesia's Ministry of Marine Affairs and Fisheries Fish Quarantine and Inspection Agency; the United Kingdom's Fish Health Inspectorate, Institute of Fisheries Management; and Japan's Food Safety and Consumer Affairs Bureau, and National Research Institute of Aquaculture Fisheries Research Agency to seek access to water quality datasets collected before, during and after outbreaks, and data/records on human health implications. In addition, all individuals interviewed were surveyed to gather information relating to human health issues/observations associated with the carp herpesvirus. No human health issues were identified by participants interviewed.

All of those interviewed indicated there had been no examples of human health issues associated with contact or consumption of water or fish carrying the carp herpesvirus. Though there have been no reported cases of human illness, irritation or condition of any form resulting from contact with the wild virus, live attenuated vaccine or infected carp, some acknowledged risk that individuals who come in contact with infected deceased fish following release of the virus may be exposed to zoonoses which may proliferate as a result of temporary degradation in water quality. Examples include bacteria such as mycobacteriosis and nocardiosis, which are capable of infecting humans, entering through abrasions and can cause abscesses, ulceration and lymphatic swelling, requiring treatment with antibiotics.

Individuals involved in the collection of deceased fish from Lake Chautauqua were reported to wear personal protective equipment including sturdy and waterproof protective gloves, boots and clothing initially to limit contact with deceased fish and liquids, however many later elected to dispense with protective equipment as it was considered cumbersome. Whilst this is not advised, no human health issues were recorded after having done so..



Those involved in clean-up activities on Lake Biwa in Japan were not believed to have worn protective equipment. No human health issues were recorded in relation to this case study.

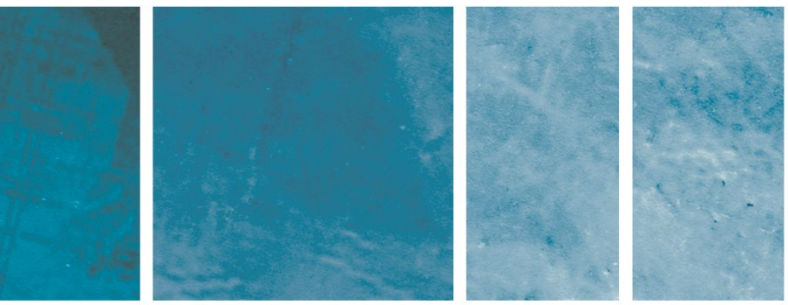
It is recommended that appropriate protective clothing be used by clean up participants in Australia to limit exposure to harmful bacteria.

Whilst risk of infection for humans is also considered to be extremely low/negligible, it is also recognised that this may be an area of concern for the Australian public. It is recommended that indepent expertise in zoonoses be engaged to inform consideration of relevant risks to human health, and inform consideration of future work required.

Appendix 1 Travel itinerary

Date	Location	Business/Organisation	Notes
15 June	Jakarta, Indonesia	Centre for Fish Quarantine	Meet with senior officers to discuss experiences with CyHv-3 in Indonesia, and seek access to unpublished datasets on human health aspects, and clean up methods employed
17 June	Indonesia	Various	Meet with regional managers and aquaculturists on Cirrata Reservoir, Blitar Province to collect information on biosecurity practices employed, clean-up methods employed following outbreak events and human health issues experienced
23 June	United States	Chautauqua Lake Association	Meet with Association President to learn about methods used for clean up in 2004, detailed costings, effectivness, human helath issues and resources involved
24June	United States	New York State Federation of Lakes	Meet to discuss and gain access to water quality data collected before, during and after clean-up efforts. Also discuss angler-based data collection for use in evaluating carp population recovery
29 June	United Kingdom	CEFAS [Centre for Environment, Fisheries and Aquaculture Science] Weymouth Laboratory	Meet with head of the Fish Health Inspectorate, the official service for the control of serious diseases of aquatic animals in England and Wales. Meet with OIE [World Organisation for Animal Health] expert on KHV disease diagnosis and epidemiologists looking into the spread of infection
30June	United Kingdom	Various	Visit large koi retail businesses to understand impacts of the virus on their business and biosecurity measures employed to mitigate impacts
2 July	United Kingdom	The Angling Trust	Speak with the peak recreational fishing organisation about how the virus has impacted on their industry and biosecurity measures being employed to limit spread of the virus

Date	Location	Business/Organisation	Notes
6 July	Israel	Dag noy Ornamental Fish	Visit one of the largest distribution and breeding company, specialising in koi and goldfish. Visit a facility that vaccinates all their fish to understand its use, benefits risks, effectiveness and duration in more detail
7 July	Israel	Hezorea Aquatic	Visit a large koi breeding facility that does not vaccinate their fish, but has successfully employed thorough biosecurity measures to prevent infection. Biosecure facilities make up 15 per cent of Israel's koi exports.
8 July	Israel	Ma'agan Michael	Ma'agan Michael is one of the largest fish farms in Israel and produces large quantities of food-fish for export and for the local industry, including carp. Visit a large operation which utilises vaccine to manage KHV, to understand its use, benefits risks, effectiveness and duration in more detail
9 July	Israel	Kovax	Meet with developers of the vaccine. Establish progress in development of an inactive vaccine. Explore options for importation of 'live' attenuated vaccine. Develop understanding of vaccine effectivness (duration)
13 July	Japan	Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries (MAFF)	Meet with those responsible for conducting KHV disease control in Japan, to seek information of KHV infection occurrence throughout Japan including cases in farmed and wild carp and koi.
14 July	Japan	Research Institute for Humanity and Nature	Discuss ecological impacts of CyHV-3 with researchers who have been undertaking work on effects of KHV disease on ecosystem functions such as material cycling to understand losses associated with KHV including ecosystem services and other ecological/cultural phenomena
14 July	Japan	Site visit	Site visit to Lake Biwa to gather accounts and observations from local operators and community members.



Date	Location	Business/Organisation	Notes
15 July	Japan	Freshwater Branch Office, Ibaraki Fisheries Research Institute	Gather information of recent status of KHV infection in Kasumigaura Lake and observe heat ramping method. It is understood that carp aquaculture for food consumption has recommenced, however production is reported to be half of that prior the KHV disease event in 2007. Carp juveniles for aquaculture take immunity against KHV by treatment with elevating water temperature after infection with KHV through supply of the lake water containing the virus
15 July	Japan	Site visit	Visit one of earliest infection sites where more than half of Japan's farmed carp are produced. Initial infection was in October 2003 when water temperature in the lake was 16–18°C (Sano et al., 2004a), which resulted in mass mortality of an estimated 1200 metric tonnes of common carp cultured in the lake
16 July	Japan	Niigata Koi breeders, and the Niigata Prefectural Freshwater Fisheries Experimental Station	Niigata is the origin of Nishikigoi [decorative coloured varieties of carp] and roughly 70 per cent of koi breeders are gathering in the region so KHV is one of the most important issues. Visit leading Nishikigoi breeders and manager of Niigata Prefectural Freshwater Fisheries Experimental Station, who have helped to develop strict guidelines to manage KHV risks
17 July	Japan	National Research Institute of Aquaculture (NRIA) Fisheries Research Agency	NRIA undertake national disease control, technical support and disease diagnosis. Meet with Head of Disease Diagnosis Group of Diagnosis and Training Center for Fish Diseases who is in charge of a Reference Laboratory for KHV disease in OIE

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