NSW GOVERNMENT Department of Primary Industries

OUT16/3084

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Dear Norm

Thank you for your email regarding the potential management of Common carp in Australia using a naturally occurring virus known as Cyprinid herpesvirus 3 (CyHV-3) as a biological control agent. You have raised a number of questions, which I have tried to answer in as much detail possible below.

1. Will CyHV-3 mutate and if so, what are the risks of this for non-target species?

When it comes to understanding the effects of mutations in viruses, work on many viruses over many years has resulted in some general observations. Firstly, some viruses are naturally promiscuous, meaning that they will infect a wide range of species (e.g. the influenza viruses). The group of viruses to which CyHV-3 belongs (herpesviruses) are not considered promiscuous viruses. In fact, herpesviruses are generally considered to be species-specific, i.e., each host species has its own herpesvirus(es).

Secondly, viruses that are species-specific can occasionally jump into new hosts, but these jumps seem to be determined by two factors:

1. Virus jumps really only occur between closely-related host species (e.g. AIDS virus and Ebola virus jumping from non-human primates into humans).
2. The nature of the genetic material of the virus. Viruses can be broadly classified as RNA or DNA viruses depending on the nature of their genetic material. It is well-known that DNA viruses (like CyHV-3) are relatively stable, whereas RNA viruses (like AIDS virus, Ebola virus, influenza virus) are much more likely to undergo mutations that potentially allow these viruses to jump hosts (although, as already mentioned, generally the jump is still into a closely-related species). Some small and very simple DNA viruses may jump species, but, by contrast, CyHV-3 is a very large and complex DNA virus, and such viruses are rarely associated with jumps.

It's worth noting that the two viruses that have been released in Australia to control rabbits, the calicivirus (an RNA virus) and the virus causing myxomatosis (a complex DNA virus) have been present in Australia for about 20 and 60 years, respectively. There is still no evidence at all of either virus jumping into another host during all that time.

For CyHV-3, the important observation is that it has only been found in carp, which belong to a group of fish known as cyprinids. There are no native cyprinids in Australian waterways. The native fish in Australia that are most closely related to cyprinids are the native catfish. The susceptibility of two different species of native catfish to koi herpesvirus has been tested, and there was no evidence of disease or of virus multiplication in either species.

2. Will Spring viremia of carp (SVC) be brought into Australia for the control of carp?

I am unaware of any government agencies in Australia that are currently considering importing Spring Viremia of Carp for the control of *Cyprinus carpio* in Australia. New South Wales

Department of Primary Industries is currently leading a project with funding through the Invasive Animals Cooperative Research Centre to seek legislative approval to import and use CyHV-3 as a biological control agent for Common carp. This course of action is in response to the findings of research conducted by the CSIRO over the last eight years which demonstrates that CyHV-3 only affects Common carp, and that Common carp present in Australia are highly susceptible to the disease caused by CyHV-3.

3. Does this virus present risks to humans similar to 'legionnaires disease' which is known to be in certain fertiliser products that have been shown to infect humans if not handled correctly?

It is important to note that CyHV-3 and legionnaires disease, and the risks that they respectively present to humans are extremely different. CyHV-3 is a species-specific herpesvirus which has been shown to only affect Common carp. Legionnaires disease is a form of atypical pneumonia caused by inhalation of a bacterial pathogen of amoebae and humans.

There are multiple lines of evidence demonstrating that CyHV-3 will not infect humans:

- The virus has been described since the 1990's and is now present in over thirty-two countries worldwide. Fishers, aquaculturists and Koi enthusiasts come into contact with the virus on a regular basis within affected areas, and no adverse effects have been documented.
- A significant though unquantified proportion of carp sold internationally are vaccinated with a weakened strain of the virus, and are then sold for human consumption. No human health concerns have been raised in relation to the significant amount of vaccinated carp consumed every year.
- Carp aquaculturists in some countries harvest farmed carp immediately upon observing clinical signs of CyHV-3 and sell infected fish for human consumption at a reduced price. Despite this no human health concerns have been raised in relation to human consumption.
- There are no documented instances of closely related viruses such as carp pox virus (CyHV-1), or Goldfish hematopoietic necrosis virus (CyHV-2) causing issues for humans.
- Researchers from CSIRO have tested mice as a model mammal species and confirmed that the virus did not replicate within inoculated mice.
- Researchers in Israel attempted to infect mammalian cell lines including a rat cell line, a human cell line, and a monkey origin cell line with CyHV-3, and did not record evidence of viral invasion or the presence of virus.
- During a recent study tour of the United States, United Kingdom, Israel and Japan all fishers, aquaculturists, researchers, fisheries managers and community groups surveyed confirmed that they never experienced or witnessed any health issues, including respiratory, skin, eye or oral irritation/sensitisation as a result of contact with the virus in either its wild or attenuated form.

4. How will Koi breeders be able to protect their businesses and fish stocks from the virus if approval is granted for release of CyHV-3 for the control of carp?

Whilst it is proposed to seek approval to import and release CyHV-3 for the biological control of carp, it is important to note that there is still a long process yet to be undertaken before biological control of carp might be possible in Australia. Importantly, there is a need to undertake a thorough risk assessment process to explore any risks that might arise through use of the virus, and strategies to overcome them. There is also a need to undertake extensive stakeholder consultation to establish the views of the broader community and key stakeholder groups on this issue.

If CyHV-3 is eventually approved for use for the control of carp in Australia it will be important that Koi breeders, retailers and enthusiasts employ effective biosecurity measures to protect their fish. Fortunately, consultation with Koi breeders and hobbyists in the United Kingdom, United States, Israel and Japan has confirmed that adoption of basic biosecurity practices including sourcing fish from disease free origins, quarantining of new fish with sentinel fish at permissive temperatures,

sterilisation and disinfection of hatchery equipment, and treatment of water sources to ensure freedom from the virus are highly effective in protecting captive Koi populations from CyHV-3.

NSW Department of Primary Industries will be consulting with Koi breeders and enthusiasts to develop a biosecurity plan to help them to safeguard their businesses and fish from the virus, in the event approval is granted for its use as a biological control agent for carp.

5. How sure are we that CyHV-3 is species specific? And why have only 13 fish native fish species been tested to confirm species specificity?

On the basis of research undertaken by CSIRO over the last 8 years we are very sure that CyHV-3 is highly species specific, and only causes disease in Common carp.

Testing of CyHV-3 in the high-security Fish Diseases Laboratory at the Australian Animal Health Laboratory (AAHL) in Geelong, Victoria has proven that the virus won't affect native Australian or important introduced species of fish. It has been shown to pose no danger to 13 native species such as Murray cod, various species of perch, eel and catfish, as well as a crustacean (yabbies) and a non-native fish species, the rainbow trout. This research has shown that there are no clinical or pathological changes in these non-target animals, nor is there any evidence that the virus multiplies in these species.

Chickens, mice, frogs, turtles, yabbies and water dragons have also been tested as representatives of a wider community of birds, mammals, amphibians, crustacea and reptiles. Again the virus has shown no effect on them.

It is important to note that it is not necessary to test every single species to assess risk of infection from a virus. This is because the taxonomic relatedness of a non-target species to the natural host is a key factor in determining whether a virus can cross a species barrier. Consequently, if non-target species of high taxonomic relatedness to the natural host species are shown to be unable to develop the disease caused by CyHV-3, it follows that more distantly related species will also be unlikely to develop the disease.

Selection of non-target species to be tested by CSIRO was based primarily on taxonomic relatedness to the natural host species (carp), but also on non-target species having the potential to be exposed to water bodies containing CyHV-3-infected carp. Figure 1 shows the relationships between taxonomic groupings of fish, and highlights groupings included in non-target species testing conducted by CSIRO (in blue). In the absence of any native cyprinid species in Australia, the most closely related taxonomic grouping with representatives in Australian waterways is the Order siluriformes. Two species of siluriformes were tested: Blue catfish (*Neoarius graeffeii*) and Eel-tailed catfish (*Tandanus tandanus*). An anguilliform was also tested (Short-finned eel, *Anguilla australis*), because the virus anguillid herpesvirus 1 is also a member of the Cyprinivirus genus (which includes CyHV-3, and closely related CyHV-1 and CyHV-2). Representative Perciformes that were tested included Murray cod (*Maccullochella peeli*), Golden perch (*Macquaria ambigua*), Silver perch (*Bidyanus bidyanus*), Olive perchlet (*Ambassis agassizii*) and Carp gudgeon (*Hypseleotris klunzingeri*), while Common jollytail (*Galaxias maculatus*) and Australian smelt (*Retropinna semoni*) were selected as representative osmeriformes. Atheriniform Crimson-spotted rainbowfish (*Melanotaenia duboulayi*), mugiliform Sea mullet (*Mugil cephalus*), and clupeiform Bony bream (*Nematalosa erebi*) were also tested. An economically important introduced salmoniform (Rainbow trout, *Onchorhynchus mykiss*) was tested. A petromyzontiform Lamprey (*Mordacia mordax*), a crustacean (*Cherax destructor*), two amphibian species, Peron's tree frog (*Litoria peroni*) and Spotted marsh frog (*Lymnodynastes tasmaniensis*), two reptilian species, Australian water dragon, *Intellagama lesueurii*) and Macquarie short-necked turtle (*Emydura macquarii*), chickens (*Gallus gallus domesticus*) and lab mice (*Mus musculus*) were also tested.

Of the 10 orders of bony fishes that occur in freshwater or estuarine habitats in Australia (potentially allowing contact with carp), the susceptibility of representative species from seven of these orders was tested. No evidence of viral replication or disease was observed in any of the 7 orders examined, nor in any other non-target species that was tested. The absence of virus replication is particularly important because not only does it explain the lack of acute disease in non-target species, but it also precludes any potential chronic effects by the virus on the health of

non-target species (e.g. impacts on growth, breeding, feeding or fitness). Clearly, if CyHV-3 is unable to replicate (and survive) within a non-target species, it cannot cause either acute or chronic (long-term) effects in those species.

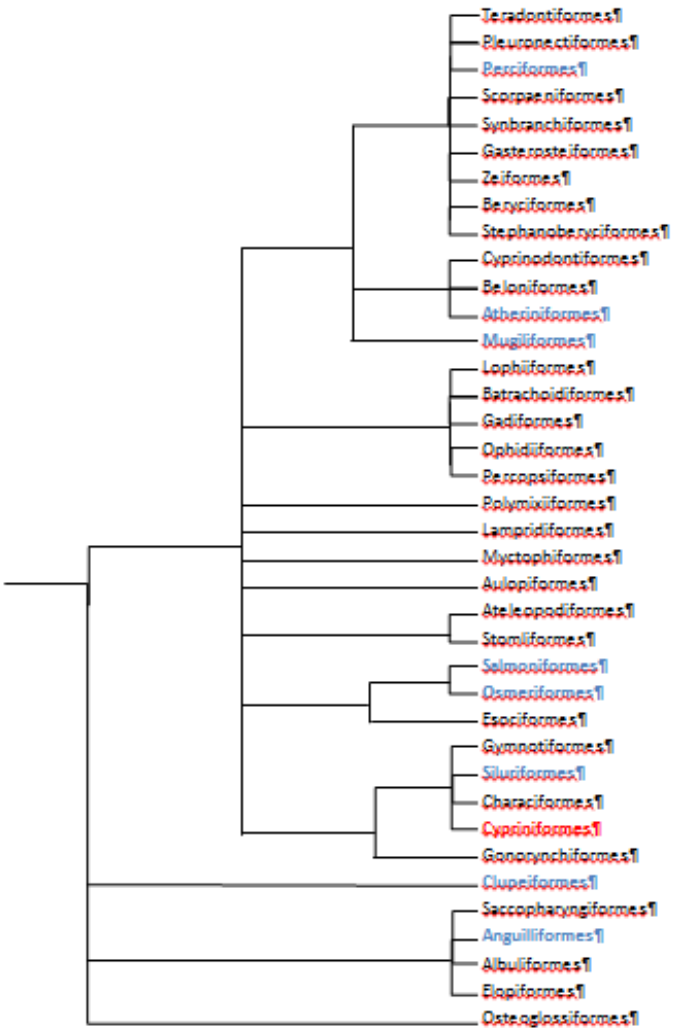

Figure 1 Taxonomic groupings of bony fishes. Orders coloured blue were included in non-target species testing. Cypriniformes (red) is the order within which the target species (Common carp) is classified (based on "Fishes of the World" by Joseph Nelson).

6. Can birds transfer CyHV-3 on their feet/feathers to other waterbodies? If so, does this present a risk to nearby countries?

The risk of CyHV-3 being transferred between waterbodies via birds is likely to be remote, particularly to nearby countries. During a recent study tour NSW DPI staff visited numerous recreational fisheries in the United Kingdom that had been affected by CyHV-3 outbreaks since 1998 when the virus was first identified. Many of the affected fisheries were adjacent to water bodies that had never experienced a CyHV-3 outbreak despite being separated only by 3-4 metres of grassy embankment with populations of waterfowl moving between waterbodies in an unrestricted manner.

7. Rabbit biocontrol using Myxomitosis and Calici was unsuccessful – why will biocontrol using CyHV-3 be any different?

Australia's biocontrol program using Myxoma virus in 1950 and the Rabbit Calicivirus in 1995 has been extremely successful in drastically reducing pest rabbit numbers in Australia. Rabbit biocontrol in Australia is actually considered one of the greatest success stories of biological control in the world. Myxomatosis and Calicivirus still limit rabbit numbers to about 15% of their potential numbers in Australia, and it's estimated that the benefit of rabbit biocontrol to agriculture is worth more than A$70 billion.

With that said, it is true that host-pathogen co-evolution led to a less severe form of Myxomitosis which allowed rabbit numbers to increase after release of Myxomitosis. However Calicivirus was successful in significantly reducing rabbit numbers once again and in keeping rabbit numbers low for over a decade.

In contrast to Myxomavirus, a reduction in Calicivirus virulence has so far not been observed. However, evidence for developing resistance in some Australian wild rabbit populations has now been described, and rabbit numbers are again on the rise. This case study highlights the need to not solely rely on biological control to manage pest rabbits, but to always combine it with conventional control methods. The same strategy must also be employed in the management of carp in order to ensure desired outcomes are delivered.

8. Cat biocontrol on Marion Island using parvovirus was unsuccessful, why will biocontrol using CyHV-3 be any different?

It is important to note that the focus of Australia's carp biocontrol program is to reduce impacts of carp by dramatically reducing their abundance in Australian waterways. Eradication of carp in Australia is not considered to be a realistic goal.

Though the viral disease feline panleucopenia, also known as feline parvovirus, was effective in dramatically reducing the population of cats on Marion Island after its release in 1977, feral cats were not fully eradicated from the island until 1992 after an integrated campaign of trapping, baiting and shooting[1].

Similar to the case study of cat biocontrol on Marion Island using feline parvovirus, the use of CyHV-3 by itself is unlikely to cause eradication of carp in Australia. There will be a need to employ a range of measures to supress carp populations and promote recovery of native fish populations in order to ensure long-term reduction of the impacts caused by carp in Australian waterways.

9. Will release of CyHV-3 result in establishment of the carp virus, and resistant strains of carp which will then flourish?

Studies have shown that CyHV-3 will survive for approximately three days in water without a host, and so if carp were completely removed from a waterway the virus would not be expected to persist. However, a key characteristic of herpesviruses is their ability to establish and maintain a latent state in their host and reactivate following cellular stress. Consequently, any carp that survives infection will become persistently infected and can excrete infective virus for at least 10-15 years after exposure. This is actually a positive outcome with respect to carp biocontrol as it provides a mechanism through which the surviving carp population may continue to be infected by the virus.

Though available evidence suggests that the carp Virus would be effective for managing carp in Australia, it is possible that carp may develop immunity to CyHV-3 over time. To ensure that Australia's carp biocontrol program is successful (if approved), it will be important to not rely solely on use of CyHV-3 to control carp, and to employ a range of other techniques at the same time to control carp and support recovery of native fish species. We know from research conducted in the Murray-Darling Basin that carp are less successful invaders within healthier river reaches. Armed with this knowledge, there is a significant opportunity to intensify efforts to rehabilitate Australian

[1] http://www.ceru.up.ac.za/downloads/A_review_successful_eradication_feralcats.pdf

waterways whilst undertaking carp biocontrol, making it much harder for carp to repopulate after release of the virus.

Continued efforts to supress carp numbers through carp fishing events, excluding them from important breeding habitats using screens, commercial fishing for carp and use of other methods will further assist. There may also be a need to invest in research to develop and test new and more effective strains of CyHV-3 to combat immunity. This approach is currently being employed for rabbits in an effort to stop them from further damaging Australia's agricultural and natural environment.

10. If the virus is released this will require a huge clean-up effort initially, and continued clean up effort each spring as carp kills re-occur. How will this be undertaken?

If approval is given to use the carp virus for the biological control of carp in Australia, it will be vital to ensure there is an effective clean-up plan in place to minimise impact on water quality, and protect native species. This will be a significant task, requiring input and assistance from all levels of government, as well as a range of organisations and community groups. Over the next 12-18 months the NSW Department of Primary Industries will be developing a clean-up plan in partnership with stakeholders. There will be significant investment required to undertake clean-up activities, and discussions are underway with potential partners to secure investment required.

11. A single biological control agent may not be effective in eliminating the carp and the carp virus. What back up measures are proposed if CyHV-3 does not eradicate carp completely?

Though available evidence suggests that CyHV-3 will kill >70% of carp if approval is granted to release the virus, it is important to note that this single intervention will be unlikely to cause eradication of carp in Australia. As addressed previously in answer 9, it will be vital that a range of complimentary measures be employed at the same time to control carp and support recovery of native fish species. There may also be a need to invest in research to develop and test new and more effective strains of the carp virus to combat immunity. This approach is currently being employed for rabbits in an effort to stop them from further damaging Australia's agricultural and natural environment.

It is also important to note that the focus of Australia’s carp biocontrol program is to reduce impacts of carp by dramatically reducing their abundance in Australian waterways. Eradication of carp in Australia is not considered to be a realistic goal.

12. What happens to the virus once carp are removed from a water body? Does it die off or remain in the water forever?

Studies have shown that CyHV-3 will survive for approximately three days in water without a host, and so if carp were completely removed from a waterway the virus would not be expected to persist.

13. Because temperature impacts on effectiveness of CyHV-3 will this prevent its use in the Murray-Darling Basin?

Because temperature is known to impact on effectiveness of CyHV-3 it will be vital that the virus is released during optimal conditions to maximise carp mortalities. CSIRO are currently conducting computer modelling which uses data collected on the virus, the target species (carp), and environmental information to help determine how the virus should be released in order to maximise the impact on carp populations (if approval is granted to do so). This modelling will then provide a basis for development of a strategy to guide release of the virus to maximise effectiveness.

14. How will the virus be released?

It is important to note that the carp virus is not currently approved for use in Australia, and there is still a long process yet to be undertaken before biological control of carp might be possible in Australia. Importantly, there is a need to undertake a thorough risk assessment process to explore any risks posed by use of the virus, and strategies to overcome them. There is also a need to

undertake extensive stakeholder consultation to establish the views of the broader community and key stakeholder groups on this issue.

If the virus is eventually approved for use for the control of carp in Australia, it will likely be introduced into Australian waterways by catching and infecting a number of carp, and then releasing them to enable the virus to be transmitted to the rest of the wild carp population.

Computer modelling work currently being undertaken by CSIRO (see response to 14 above) will help to determine whether the optimal release strategy involves release of the virus across the Murray-Darling Basin in a synchronised introduction, or in a staged approach.

15. Would Genetic manipulation be a better control technique for carp in Australia?

There is interesting research demonstrating that synergistic use of carp biocontrol and gene technology is the best approach to reduce carp biomass in the long term[2]. Genetic manipulation by itself is unlikely to be effective for the control of carp in Australia as it would require stocking of a prohibitive number of genetically modified carp to enable distortion of the sex ratio of the overall carp population. However release of CyHV-3 would enable the population size of carp to be significantly reduced, which would then enable genetic technology to distort the sex ratio with lower levels of carp stocking.

Once again Norm I would like to thank you for taking the time to write and raise your concerns. Please feel free to contact Mr Matt Barwick, who is managing the carp Biocontrol Program for NSW DPI on matt.barwick@dpi.nsw.gov.au or 0422 752 789 if you would like to discuss in more detail.

Yours sincerely

Bob Creese
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15/02/2016

[2] http://www.publish.csiro.au/paper/MF13117.htm