Summary of scientific concerns about uncertainties in understanding about carp biocontrol using herpesvirus CyHV-3.

Briefing prepared for the Senate Rural and Regional Affairs and Transport Legislation Committee, 22 June 2018.

Dr Jonathan Marshall

Aquatic Ecologist
jonathan.marshall@des.qld.gov.au

1. Introduction

The Federally funded National Carp Control Plan (NCCP) is considering the feasibility for biocontrol of carp in Australia using carp herpesvirus CyHV-3 in order to restore native freshwater biodiversity.

It is well recognised that invasive carp (*Cyprinus carpio*) can harm native ecosystems (e.g. Vilizzi et al., 2015), and the concept of carp biocontrol certainly warrants rigorous scientific consideration. However, this summary document discusses uncertainties concerning the generality of the scientific understanding of the damage carp cause, the potential effectiveness of carp herpesvirus as a biocontrol measure, and possibilities of unintended negative outcomes from its deployment.

Since December 2016, the Australian Government has invested $5 million into research to support development of the NCCP, and this research is addressing several important knowledge gaps.

Research funded though the NCCP is currently examining carp biomass, viral epidemiology, water quality risks, carp clean-up options, social attitudes and impacts, and expected ecological benefits of carp biocontrol, among many other projects. It is not my want or intention to criticise the research programme of the NCCP or pre-empt results of the research itself. However, despite this research, I feel that large uncertainties in understanding will remain and critical scientific knowledge gaps will still require additional research by the time the NCCP is required to make its recommendation in late 2019. These are mostly owing to the limited time and funding available, and also the nature of cutting-edge research itself – whereby further complexities and uncertainties are uncovered as the research proceeds. My view is that the magnitude of the uncertainties and risks as they currently stand warrant a longer term research programme beyond the current NCCP commitments.

Key uncertainties and knowledge gaps which I consider to be important for informing a decision about using carp herpesvirus for biocontrol of carp are explained below, and can be summarised into three themes:

- Uncertainty of efficacy of carp biocontrol using CyHV-3;
- Uncertainty and lack of knowledge of benefits to native freshwater biodiversity; and
- Lack of knowledge of risks of unintended impacts to native biodiversity.

If these issues are not adequately addressed, remaining uncertainty would mean that the intended ecological benefits could actually be minor, and unintended impacts could be major. Therefore, it is imperative that the science to inform this decision is comprehensive in relation to the complexity of the question, is defensible and is quality assured via the standard, robust, scientific peer-review process. Because of the potential risks and irreversibility of introducing a virus into Australia, the onus should be on the science to provide robust evidence that risks will not manifest and that the desired benefits will be realised. At present I do not believe the science is comprehensive enough to do this.

Some of the uncertainty and knowledge gaps explained below are general, but others are more specific to the ecology of the dryland-rivers of the northern Murray Darling Basin (MDB) in Queensland and New South Wales, where the majority of my relevant professional career has focussed. This region represents approximately 40% by area of the MDB and is characterised by semi-arid climate, high summer temperatures, intermittent river flow regimes where rivers dry to a series of waterholes, and a relatively sparse human population density. These attributes combine to pose particular concerns around the potential release of carp herpesvirus in the Northern MDB.

2. Uncertainty of efficacy of carp biocontrol by herpesvirus CyHV-3

a. The absence of carp herpesvirus CyHV-3 in Australian carp populations remains uncertain: Herpesvirus infections can be very hard to find in wild (outbred) hosts (Boutier et al., 2015) and may persist at a low level, unable to be detected and only causing disease when other factors such as temperature and population density align. While CyHV-3 hasn't been isolated in wild Australian carp (McColl et al., 2016), this does not means its absence is certain. Indeed, many of the reported isolations from elsewhere in the world were only able to be detected because of amplified disease states, which manifest only under specific conditions (e.g. where animals are immunocompromised owing to inbreeding, over-crowding, or co-infection; or where water temperatures and animal behaviour is particularly favourable to viral infection and subsequent outbreak) (Boutier et al., 2015). If the CyHV-3 strain is already present in Australia, there may already have been evolution of resistance to it in Australian carp (Marshall et al., 2018) (see below). This is currently a critical knowledge gap.

b. Lack of knowledge around genetic effects on virulence of carp herpesvirus to Australian wild carp populations: The high rates of mortality caused by carp herpes virus in outbreaks overseas affected populations of carp in aquaculture-type conditions, where animals were likely stressed (immunocompromised) and inbred (Boutier et al., 2015). Wild carp populations, such as in Australia, have been shown to be much less vulnerable (Thresher et al., 2018). It is currently unknown to what extent the genetic variability (polymorphisms) in Australian carp may confer resistance, or to what extent known resistance conferring genes exist in Australian wild carp populations.

c. Water temperature effects: Optimal water temperature for the CyHV-3 virus replication and infection is 15 – 25^{o}C, it causes disease (i.e. sickness and morality) between 18 – 28^{o}C, and above 30^{o}C the virus ceases to replicate and cannot infect. In general, disease from CyHV-3 herpesvirus is less severe at the population level if water temperature is not optimal for virus replication. Furthermore, exposure of infected carp to temperatures in excess of 30^{o}C has been used overseas to immunise carp against the virus. In this situation, the virus dies in the infected animal, the animal does not exhibit symptoms, and the animal develops antibodies against the virus. Individuals immunised in this way are resistant to future mortality from that viral strain (Boutier et al., 2015). In the northern MDB, water temperatures are in excess of 30^{o}C for on average 5-6 months per year, whereas permissive temperatures occur for only one or two months each year. Therefore, if the herpesvirus is released and infects some carp populations when water temperature is high, these populations would be expected to be immunised by the high temperature, and avoid mortality. Furthermore, infected carp adopt behaviour termed 'behavioural fever' whereby they actively seek hot water (>30^{o}C) microhabitats, increasing the prevalence of the temperature immunisation effect (Rakus et al., 2017). Given this, it seems unlikely the virus will be an effective biocontrol agent in warmer regions of Australia, especially during the summer period when carp aggregations in waterholes may be targeted for virus release. Indeed, release of the virus at this time of year could have the opposite effect and cause population level immunisation rather than mass mortality. The work currently underway in the NCCP will go some way to address aspects of these knowledge gaps, however critical uncertainties will remain regarding virus ecology, interaction with carp behaviour, unpredictable river flow, and high temperatures in the northern MDB.

d. Unknown potential for evolution of viral resistance in Australian carp populations: There is uncertainty about how quickly naïve wild carp populations will develop resistance to the carp herpesvirus. Evidence from herpesvirus outbreaks in North American wild carp suggests that a single 'knock-down' mortality event may be all that is required to confer population resistance, with no detectable carp population reduction in subsequent years (Thresher et al., 2017). Further research is required to understand the evolution of resistance in Australian wild carp and the potential for use of new viral strains to counter resistance.

e. Doubt about biocontrol of carp at the population and landscape scale: Observations of overseas outbreaks of carp herpes virus in wild carp populations suggest that in most cases mortality is highly localised (e.g. within a lake or pond), short lived (i.e. within a season), not repeated when similar conditions are subsequently met, and in most cases there is no long-term reduction in carp population abundance following mortality events (Thresher et al., 2017). Given the high mobility of carp in Australian rivers (as indicated by the rapidity of their invasion) and their high reproductive capacity (fecundity), localised mortality events of the types described from overseas seem unlikely to perpetuate population-scale biocontrol of carp in Australia. There is a need for additional carp population research and modelling at the large spatial scales over which carp populations function in order to better understand and predict biocontrol outcomes.

f. Lack of research into integrated secondary control measures: if herpesvirus does work as a valid biocontrol measure and successfully reduces abundance of carp at population scales, this effect will likely be short lived due to potential evolution of resistance described above. Effective secondary carp control measures and complementary actions to enhance native biodiversity benefits (such as habitat restoration, re-snagging, appropriate land management practises, flow restoration, longitudinal population connectivity, and possibly restocking), would need to be developed and ready for use when needed. Without the potential benefits of secondary control measures, the utility of the virus will be transitory. To release the virus without these measures primed for follow-up application would waste the opportunity the virus may proffer. At present such secondary control measures are being considered at a conceptual level only by the NCCP.

3. Uncertainty and lack of knowledge of benefits to native freshwater biodiversity from carp viral biocontrol

a. Simplistic understanding of carp and turbidity: many of the purported benefits from successful carp biocontrol stem from the assumption that reducing carp abundances will proportionally reduce the turbidity ('muddiness') of waterbodies. This widely publicised view (see http://www.carp.gov.au/The-Carp-Problem) ignores that large regions of the MDB (e.g. lowland parts of the northern MDB) naturally have high turbidity because of their floodplain soil properties and local sediment type. Furthermore, many palaeoecology studies have demonstrated large increases in the turbidity of MDB floodplain wetlands following early European settlement and changes in landuse (e.g. Gell and Reid, 2014). These occurred well prior to the introduction and proliferation of carp in the 1970s. While carp certainly can increase turbidity in some situations (e.g. King et al, 1997), reversal of elevated turbidity by removal of carp is not universally likely (or even possible) because carp may not be the major driver of high turbidity in many settings. Additional research is needed to better understand and model the likely effects of carp reductions on turbidity and interactions with other drivers of high turbidity across a broad spectrum of settings in which carp occur in Australia.

b. Macroinvertebrate biodiversity (shrimps, crayfish, insect larvae etc): Some Australian studies (in the southern MDB) have demonstrated a decrease in richness of benthic invertebrates, caused indirectly by carp (Vilizzi et al., 2014). But these observations are limited to few Australian settings and should not be considered universal. For instance, I am currently analysing monitoring data from northern MDB and adjacent rivers with and without carp present. This indicates little detectable difference in macroinvertebrate composition or abundance due to carp presence or abundance. There is little evidence to suggest ecological benefits to macroinvertebrate biodiversity in the northern MDB from carp biocontrol. The exception is a river snail (*Notopala* sp.) which was found to be absent from all sites in rivers with carp present and occurred at all sites in rivers without carp. These results suggests recovery and repopulation of *Notopala* snails would require carp elimination and an active reintroduction program as species within the group are all very poor dispersers (Carini and Hughes, 2006). These differing invertebrate responses to carp in different Australian settings demonstrate the ecological complexities that must be understood when attempting to quantify the benefits of carp biocontrol. At present ecological benefits is being addressed within NCCP by an expert elicitation process, but in my view such a process can be useful for hypothesis generation, but cannot compensate for a lack of data. More research into this issue is needed.

c. Native fish biodiversity: The relative contributions to biodiversity loss from carp compared to other current ecosystem stressors (e.g. barriers to movement, habitat loss/degradation, and hydrological change), which may impact native fish biodiversity, are poorly understood. Benefits from reducing carp abundance are thus also uncertain, and as this is one of the principle aims of the NCCP, it is an important knowledge gap requiring additional research.

4. Uncertainty over the risks of unintended impacts to native biodiversity from carp biocontrol by herpesvirus

a. Anoxia from decomposing carp: Preliminary NCCP experimentation suggests that anoxia (zero dissolved oxygen in water) is likely from carp deaths above a threshold density. This threshold and the carp densities in different settings around Australia are current research topics for the NCCP, but large uncertainties will inevitably remain. This is of particular concern in the northern MDB where many rivers have intermittent flow and for much of the time fish are confined to isolated, stagnant, in-channel waterholes. These waterholes are naturally low in oxygen and further reductions could be catastrophic to local river biodiversity. Key uncertainties concern the impacts of anoxia on local (waterhole) populations of native fish and invertebrates, and the impacts of waterhole-scale losses of native fish on regional native fish population viability.

b. Ecosystem impacts: carp can represent a large proportion of the biomass of aquatic organisms in MDB rivers. There are currently no ecological studies or models developed to predict what will happen to river ecosystems if a large proportion of this biomass is removed (see Kopf et al., 2017). The outcomes could be positive (e.g. more native fish biomass) or negative (e.g. more nuisance algae, expansion of other invasive pest species), but which is uncertain.

c. Risks of carp herpesvirus causing disease and mortality in native species can be considered to be negligible because herpesviruses in general are highly host-specific, there is a large international body of literature confirming this specifically for carp herpesvirus, and the Australian research conducted by CSIRO has further confirmed this for a spectrum of Australian native species. Carp herpesvirus can infect a wide range of species, which can then act as virus reservoirs and transmit it to naïve carp, but carp herpesvirus only causes mortality in carp and carp x goldfish hybrids (Boutier et al., 2015).

5. Conclusions

To increase knowledge and reduce uncertainty, in order to provide the basis for a well informed decision to either introduce carp herpes virus or not, requires more time and a greater research investment than has so far been provided to the NCCP. There needs to be a greater focus on landscape-scale research relevant to the scales that Australian carp populations function and focus on the important issues raised here as well as others that are recognised by other scientists or yet to emerge. This is a highly complex domain.

It is important to recognise that the impacts of carp on Australian native biodiversity are relatively static, carp having expanded to their major current range decades ago. The problem is not worsening. Likewise, the potential of the carp herpesvirus to provide a biocontrol solution would not diminish if the research and decision time frames were to be extended. Large problems such as this require large investment and sufficient time for the science to be conducted to a satisfactory level that satisfies the standard scientific process of peer review. Releasing a virus into Australia is irreversible and while the ecological and social benefits could be tremendous, the potential for unintended harm is also large. My view is that the magnitude of the uncertainties and risks warrants a longer term research programme beyond the current NCCP commitments.

6. References

Boutier, M., Ronsmans, M., Rakus, K., Jazowiecka-Rakus, J., Vancsok, C., Morvan, L., Peñaranda, M.M.D., Stone, D.M., Way, K., van Beurden, S.J. and Davison, A.J., 2015. Cyprinid herpesvirus 3: an archetype of fish alloherpesviruses. In Advances in virus research (Vol. 93, pp. 161-256). Academic Press.

Carini, G. and Hughes, J.M., 2006. Subdivided population structure and phylogeography of an endangered freshwater snail, *Notopala sublineata* (Conrad, 1850)(Gastropoda: Viviparidae), in Western Queensland, Australia. Biological Journal of the Linnean Society, 88(1), pp.1-16.

Gell, P. and Reid, M., 2014. Assessing change in floodplain wetland condition in the Murray Darling Basin, Australia. Anthropocene, 8, pp.39-45.

King, A. J., A. I. Robertson, and M. R. Healey. 1997. Experimental manipulations of the biomass of introduced carp (*Cyprinus carpio*) in billabongs: impacts on water-column properties. Marine and Freshwater Research, 48(5): 435. https://doi.org/10.1071/MF97031.

Kopf, R.K., Nimmo, D.G., Humphries, P., Baumgartner, L.J., Bode, M., Bond, N.R., Byrom, A.E., Cucherousset, J., Keller, R.P., King, A.J. and McGinness, H.M., 2017. Confronting the risks of large-scale invasive species control. *Nature ecology & evolution*, *1*, p.172.

Marshall, J., Davison, A.J., Kopf, R.K., Boutier, M., Stevenson, P. and Vanderplasschen, A., 2018. Biocontrol of invasive carp: risks abound. Science, 359(6378), pp.877-877.

McColl, K.A., Sunarto, A., Slater, J., Bell, K., Asmus, M., Fulton, W., Hall, K., Brown, P., Gilligan, D., Hoad, J. and Williams, L.M., 2017. Cyprinid herpesvirus 3 as a potential biological control agent for carp (Cyprinus carpio) in Australia: susceptibility of non-target species. Journal of fish diseases, 40(9), pp.1141-1153.

Rakus, K., Ronsmans, M., Forlenza, M., Boutier, M., Piazzon, M.C., Jazowiecka-Rakus, J., Gatherer, D., Athanasiadis, A., Farnir, F., Davison, A.J. and Boudinot, P., 2017. Conserved fever pathways across vertebrates: a herpesvirus expressed decoy TNF-α receptor delays behavioral fever in fish. Cell Host & Microbe, 21, pp.244-253.

Thresher, R.E., Allman, J. and Stremick-Thompson, L., 2018. Impacts of an invasive virus (CyHV-3) on established invasive populations of common carp (Cyprinus carpio) in North America. Biological Invasions, 20(7), pp.1703-1718.

Vilizzi, L, Thwaites, L. A, Smith,B, B., Nicol,J, M., and Madden, P. 2014. Ecological effects of common carp (*Cyprinus carpio*) in a semi-arid floodplain wetland. Marine and Freshwater Research 65(9): 802. https://doi.org/10.1071/MF13163.

Vilizzi, L., Tarkan, A.S. and Copp, G.H., 2015. Experimental evidence from causal criteria analysis for the effects of common carp *Cyprinus carpio* on freshwater ecosystems: a global perspective. *Reviews in Fisheries Science & Aquaculture*, *23*(3), pp.253-290.