PLANNING INVESTIGATIONS **2** **2018-190**

NATIONAL CARP CONTROL PLAN

Biosecurity strategy for the koi (*Cyprinus carpio*) industry

This suite of documents contains those listed below.

NCCP TECHNICAL PAPERS

1. Carp biocontrol background
2. Epidemiology and release strategies
3. Carp biocontrol and water quality
4. Carp virus species specificity
5. Potential socio-economic impacts of carp biocontrol
6. NCCP implementation
7. NCCP engagement report
8. NCCP Murray and Murrumbidgee case study
9. NCCP Lachlan case study

NCCP RESEARCH (peer reviewed)

Will carp virus biocontrol be effective?

1. 2016-153: Preparing for Cyprinid herpesvirus 3: A carp biomass estimate for eastern Australia
2. 2018-120: Population dynamics and carp biomass estimates for Australia
3. 2017-148: Exploring genetic biocontrol options that could work synergistically with the carp virus
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
8. 2017-094: Review of carp control via commercial exploitation

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
12. 2019-176: Determination of the susceptibility of Silver Perch, Murray Cod and Rainbow Trout to infection with CyHV-3
13. 2016-152 and 2018-189: The socio-economic impact assessment and stakeholder engagement
    Appendix 1: Getting the National Carp Control Plan right: Ensuring the plan addresses community and stakeholder needs, interests and concerns
    Appendix 2: Findings of community attitude surveys
    Appendix 3: Socio-economic impact assessment — commercial carp fishers
    Appendix 4: Socio-economic impact assessment — tourism sector
    Appendix 5: Stakeholder interviews
    Appendix 6: Socio-economic impact assessment — native fish breeders and growers
    Appendix 7: Socio-economic impact assessment — recreational fishing sector
    Appendix 8: Socio-economic impact assessment — koi hobbyists and businesses
    Appendix 9: Engaging with the NCCP: Summary of a stakeholder workshop
14. 2017-237: Risks, costs and water industry response
15. 2017-054: Social, economic and ecological risk assessment for use of Cyprinid herpesvirus 3 (CyHV-3) for carp biocontrol in Australia
    Volume 1: Review of the literature, outbreak scenarios, exposure pathways and case studies
    Volume 2: Assessment of risks to Matters of National Environmental Significance
    Volume 3: Assessment of social risks
16. 2016-158: Development of strategies to optimise release and clean-up strategies
17. 2016-180: Assessment of options for utilisation of virus-infected carp
18. 2017-104: The likely medium- to long-term ecological outcomes of major carp population reductions
19. 2016-132: Expected benefits and costs associated with carp control in the Murray-Darling Basin

NCCP PLANNING INVESTIGATIONS

1. 2018-112: Carp questionnaire survey and community mapping tool
2. 2018-190: Biosecurity strategy for the koi (*Cyprinus carpio*) industry
3. 2017-222: Engineering options for the NCCP
4. NCCP Lachlan case study (in house) (refer to Technical Paper 9)
5. 2018-209: Various NCCP operations case studies for the Murray and Murrumbidgee river systems (refer to Technical Paper 8)

National Carp Control Plan - Biosecurity Strategy for the Koi (*Cyprinus carpio*) industry

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Abbreviations

CyHV-3	Cyprinid Herpesvirus 3
KHVD	Koi Herpesvirus Disease
KHV	Koi Herpesvirus
PCR	Polymerase chain reaction
NCCP	National Carp Control Program
FFVS	Future Fisheries Veterinary Service
APVMA	Australian Pesticides and Veterinary Medicine Authority
UV	Ultraviolet
TRO	Total residual oxidants
Ig	Immunoglobulin
NAAHP	National Aquatic Animal Health Program

Executive Summary

In 2019, Future Fisheries Veterinary Service developed a literature review and held two industry meetings to develop feasible biosecurity measures for the Australian koi industry against Cyprinid Herpesvirus 3 (CyHV-3), a significant disease of Koi and European carp.

Background

In recent years, CyHV-3 has been explored as a possible biocontrol measure for European carp in Australia. This virus is an OIE listed disease that at this time remains exotic to Australia. The potential release of CyHV-3 into Australian waters would introduce new risks to Australia's koi industry. The development of feasible biosecurity measures is important in preventing CyHV-3 entry into naïve populations of koi which is known to cause mass mortalities of up to 100%. This report focuses on biosecurity measures that can be applied on four different levels: incoming risks for pathogen entry onto a koi facility, risks for pathogen spread within a koi facility, outgoing risks for pathogen entry onto a koi facility, and risk of pathogen entry at a koi show/auction.

Objectives

1. Assemble best practice biosecurity strategies for the Australian koi industry for protection from CyHV-3.
2. Consult with koi industry and Government on suitability and feasibility of biosecurity options.
3. Develop biosecurity strategy for Australian koi industry in light of potential planned release of CyHV-3 including estimate of costs for Government and Industry.

Methodology

Phase 1 – Databases were searched to compile a literature review on CyHV-3.

Phase 2 – A stakeholder meeting was held in Sydney.

Phase 3 – A teleconference was held to assess the feasibility and adoptability of biosecurity measures.

Phase 4 – Suppliers, laboratories, designers, and government officials were consulted to gather estimate costs associated with feasible biosecurity options.

Phase 5 – Feasible options identified by stakeholders in previous phases of the report were compiled, and a list of recommended measures were obtained.

Results/key findings

1. Synthesis of a literature review of CyHV-3.
2. Consultation with the koi industry regarding the development of biosecurity options against CyHV-3.
3. Assessment of adoption of biosecurity options.
4. Cost estimate of strategies to industry and Government.
5. Recommended feasible biosecurity strategies and associated further research and project works suggested.

Implications for relevant stakeholders

Industry

- Access to a readily available literature review for CyHV-3.
- Compilation of feasible biosecurity measures for the industry against CyHV-3.
- Compilation of additional biosecurity measures outside of the feasible biosecurity measures that can be utilised to improve biosecurity against CyHV-3.
- Improved general biosecurity awareness for the koi industry.
- Additional education and research are likely to facilitate an improvement in biosecurity for koi keepers and farms against CyHV-3 and generally against other risks creating a lasting benefit.

Government

- Production of documents to assist government in the final assessment of the potential release of CyHV-3.
- There are key intrinsic factors of the koi industry which suggest the development and adoption of additional biosecurity measures will be challenging
- Should the koi industry adopt the many biosecurity measures outlined, then it is likely a demonstrable reduction in risk would be achieved at the individual hobbyist and farm level.
- CyHV-3 will continue to pose significant risk to the koi industry even if all the outlined feasible biosecurity measures are adopted.

Recommendations

- Further research into CyHV-3 may offer opportunities for the industry to improve biosecurity against CyHV-3.
- Development of education programs will likely increase the biosecurity awareness of koi keepers and farmers.
- Off-shore management in combination with on-arrival surveillance and quarantine of imported ornamental fish is recommended as the most effective biosecurity measure against CyHV-3.
    - The strategy of managing disease risk from offshore has been a cornerstone of Australia’s quarantine and biosecurity policy for many years, allowing Australian agribusiness to exploit its competitive production advantage and favourable market access due to freedom from many major international diseases.
- The greatest biosecurity protection that could be afforded to the koi industry is for the CyHV-3 release to not proceed, and for Australia to maintain its exotic disease status with respect to this pathogen.

Keywords

Cyprinid Herpesvirus 3, European carp, koi, Cyprinus carpio, CyHV-3, KHV, KHVD, biosecurity

Introduction

In recent years, CyHV-3 has been explored as a possible biocontrol measure for European carp in Australia. This virus has been detected in 33 countries including Canada, Indonesia, Japan, Taiwan, Thailand, Poland, Singapore, United Kingdom, and the United States of America, but at this time remains exotic to Australia.

Cyprinid Herpesvirus 3 (CyHV-3) also known as Koi Herpesvirus (KHV) is a notifiable disease in koi (*Cyprinus carpio koi*) and European carp (*Cyprinus carpio*). CyHV-3 is the pathogen responsible for causing Koi Herpesvirus Disease (KHVD) which is the damaged tissues which result from viral replication. In subclinical infections with CyHV-3, no tissue damage may be evident, hence the fish is not technically 'diseased'. Such a fish is however an infected carrier of the virus which could potentially spread the virus to other susceptible fish.

The potential release of CyHV-3 into Australian waters would introduce new risks to Australia's koi industry. The development of feasible biosecurity measures is important in preventing CyHV-3 entry into naïve populations of koi which is known to cause mass mortalities of up to 100% (R. P. Hedrick et al., 2000). Koi and carp infected with CyHV-3 can develop KHVD within the permissive range (16^{o}C to 28^{o}C) (Yuasa et al., 2008). Outside of the permissive range, transmission of the virus is known to occur in temperatures of 12^{o}C to 13^{o}C, however no clinical disease was present (Baumer et al., 2013).

Temperature can also influence the survival of carp infected with CyHV-3 as they have been shown to display signs of behavioural fever when infected with CyHV-3 and migrate towards warmer waters where KHVD is not permissive (Rakus et al., 2017). Behavioural fever has a beneficial effect on survival of carp infected with CyHV-3 compared to fish held at permissive temperature ranges (Rakus et al., 2017). However, in aquaculture and ornamental ponds and tanks, it may be difficult this utilise this behavioural response as water temperatures are rather homogenous compared to natural water ways where there are broader temperature ranges (Boutier et al., 2019). Thus, it is important to consider the feasibility and adoptability of control strategies when developing disease management strategies for the koi industry.

A recent study has highlighted the need to focus on off-shore management of diseases at the source, combined with on-arrival surveillance for preventing the risk of disease entry into Australia (Hood et al., 2019). The authors noted that historical reviews of the Australian biosecurity system did not fully manage the risks associated with ornamental fish importation. An attempt to manage biosecurity risks for the koi industry in relation to CyHV-3 if released in Australia, would appear contrary to these acknowledged best practices of managing the risk off-shore. The strategy of managing disease risk from offshore has been a cornerstone of Australia's quarantine and biosecurity policy for many years, allowing Australian agribusiness to exploit its competitive production advantage and favourable market access due to freedom from many major international diseases. To date, quarantine and ornamental fish import measures appear to have been sufficient to maintain Australia free of unplanned CyHV-3 introduction.

This report focuses on biosecurity measures that can be applied on four different levels: incoming risks for pathogen entry onto a koi facility, risks for pathogen spread within a koi facility, outgoing risks for pathogen entry onto a koi facility, and risk of pathogen entry at a koi show/auction.

Objectives

1. Assemble best practice biosecurity strategies for the Australian koi industry for protection from CyHV-3.
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3. Develop biosecurity strategy for Australian koi industry in light of potential planned release of CyHV-3 including estimate of costs for Government and Industry.

Method

Phase 1:

Search protocol for peer reviewed journal

An online database search in Scopus, Web of Science, PubMed, Science Direct Freedom collection and general search with Google Scholar and OIE website were performed to answer the research question, “What is best practice for biosecurity strategies employed to control and/or prevent CyHV-3 in Koi fish?”. The following criteria were used for each search:

- Must be an original research article.
- Full text must be available in English.
- Must be related to biosecurity, control and/or prevention.
- Must be published in a peer-reviewed journal
- Articles must be available online

The following search terms were used:

- Primary search terms: CyHV-3 OR Koi Herpesvirus
- *Subset search terms: Cyprinus carpio* OR Koi OR Carp
- Subset of subset search terms: Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*

All articles identified with the search protocol are complied. The titles of each search result were manually checked for relevance to the question. The abstracts of articles with relevant titles were checked for relevance to the question.

Grey literature

The following search criteria were used on 17/04/2019. Google and VIN were used to identify relevant grey literature articles. First twenty (20) webpage titles were scanned for relevance to the topic. Summary of relevant articles were presented in a table format.

- Full text must be available in English.
- Must be in text format
- Must be related to biosecurity, control and/or prevention.
- Articles must be available online
- Articles must not be published articles

Search terms:

- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3
- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 prevention
- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 disinfection
- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 vaccination
- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 biosecurity

Review of previous Australian studies

Previous Australian studies were supplied by the NCCP. Other previous relevant Australian studies identified during the peer reviewed article search protocol were included. The strength of the evidence (strong, adequate, weak) and risk to various sectors of the Australian Koi industry are summarised in a table (see Grey literature, below).

Review of international case studies

Email communication was made with The United Kingdom, Canada, South Africa and Singapore government officials regarding policies around the control of CyHV-3.

Additional case studies identified in the literature search were summarised.

Phase 2:

Consultation with the Koi industry

A steering committee consisting of Bradley Bradley, Gerard McDonald, Martin Rocliffe, Greg Collins, Ian Andrews, Kate McGill, Paul Hardy-Smith and Jun Hua Guo was formed. Steering committee members were invited to attend a steering committee meeting on 4 June 2019 held in Rosehill, NSW.

Details of the meeting were circulated to all steering committee members prior to the meeting. A biosecurity risk matrix was compiled to reflect potential risk pathways for CyHV-3. Biosecurity options were compiled from information gathered from the literature review performed in Phase 1 to mitigate the risk pathways identified in the matrix. These options were circulated to all stakeholders prior to the meeting. Advantages and disadvantages for each biosecurity options were listed alongside each control option.

An interactive discussion was held on the day and suitability and feasibility of each biosecurity option was discussed. Addition suggestions and comments were recorded by each stakeholder. Email communications was used to obtain additional comments from steering committee members who were unable to attend the meeting.

During the meeting, it was identified that the risk for koi farms differs drastically to the hobbyist industry. Koi farmers were contacted to discuss the feasibility and adoptability of biosecurity options. Further input was sort after from Adrian Falconer as suggested by Jamie Allnutt.

Phase 3:

Assessment of process of adoption

The biosecurity options document detailed in Phase 2 was updated with comments and suggestions by the steering committee members. The updated biosecurity options document was circulated to the stakeholders for additional comments prior to the subsequent meeting which was held by teleconference. A teleconference was held on the 25 June 2019 to discuss the feasibility and adoptability of each biosecurity option. Meeting minutes were circulated to relevant people subsequent to the meeting. Additional comments were received by email from stakeholders who were unable to attend the conference. An additional attempt to contact farms was made by email and phone.

Phase 4:

Cost estimate of strategies to Industry and Government

Feasible options identified in previous stages of the project were identified and listed in a document. Factors influencing the adoptability of the feasible options were identified. Consultation was made with equipment suppliers, chemical suppliers, graphics designers, government bodies and laboratories to obtain an approximate estimate cost to implement feasible options.

Phase 5:

Recommendations of preferred biosecurity strategy and any associated work

A series of feasible biosecurity measures identified in previous stages of the project by stakeholders were compiled and detailed against their respective risk pathways. The risk to the koi industry and individuals who keep koi after applying the risk management strategies were detailed in the risk category sections of the report.

The literature search performed in Phase 1 and comments received by the steering committee were utilised to identify areas of additional research or project work required to underpin a strategy.

Results

Phase 1:

A literature review was compiled to assemble best practice biosecurity strategies for the Australian koi industry for protection from CyHV-3 (see Appendix 3).

Identifying Relevant Peer-Reviewed Journal Articles:

Relevant articles identified with Web of Science, Scopus, PubMed, Science Direct Freedom Collection, Google Scholar, OIE website. Titles, abstracts were checked for relevance to topic manually and summary, strength of evidence compiled, and reference list of searched articles compiled. A total of 62 relevant articles were identified with the search criteria.

Database	Search terms	Number of results
Web of science on 14/03/2019	CyHV-3 OR Koi Herpesvirus	385
	Cyprinus carpio OR Koi OR Carp	381
	Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*	125
	Manual checking for relevance to topic	36

Table 1 Search results from the Web of Science on 14/03/2019.

Database	Search terms	Number of results
Scopus on 15/03/2019	CyHV-3 OR Koi Herpesvirus	298
	Cyprinus carpio OR Koi OR Carp	286
	Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*	285
	Manual checking for relevance to topic	39

Table 2 Search results from Scopus on 15/03/2019.

Database	Search terms	Number of results
PubMed on 19/03/2019	CyHV-3 OR Koi Herpesvirus	371
	Cyprinus carpio OR Koi OR Carp	359
	Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*	223
	Manual checking for relevance to topic	16

Table 3 Search results from PubMed on 19/03/2019.

Database	Search terms	Number of results
Science Direct on 26/03/2019	CyHV-3 OR Koi Herpesvirus	371
	Cyprinus carpio OR Koi OR Carp	359
	Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*	60
	Manual checking for relevance to topic	14

Table 4 Search results from Science Direct on 26/03/2019.

Identifying Relevant Grey Literature Articles:

A total of 14 relevant grey literature articles were identified.

Search terms	Number of results
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3	14,300
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 prevention	9,020
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 disinfection	6,000
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 vaccination	10,700
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 biosecurity	3,100

Table 5 Grey literature search results in Google.

A summary of the articles reviewed is detailed in Appendix 3. Overall, the grey literature provides inconsistent and details often unsupported by evidence regarding CyHV-3.

Review of previous Australian studies

Four (4) articles were supplied by the NCCP. One (1) Australian peer-reviewed article, one (1) overseas case study, and two (2) grey literature articles were obtained. One (1) additional previous Australian study was identified.

All articles and grey literature papers provided by the NCCP and additionally identified articles were summarised in a table format in Appendix 3. The summary focus only on the strength of the evidence that can assist in providing biosecurity decisions for CyHV-3 control and do not reflect the strength of the articles overall.

Review of international case studies

Government officials from the United Kingdom and Singapore responded to the enquiry. Summary of the communications are detailed in Appendix 3. Additionally, case studies from Singapore and Japan were summarised in Appendix 3.

Phase 2:

A steering committee was formed, and a meeting was held in Rosehill Bowling Club on 4 June 2019 to consult with koi industry and Government on suitability and feasibility of biosecurity options.

Consultation with the Koi industry

A steering committee consisting of Bradley Bradley, Gerard McDonald, Martin Rocliffe, Greg Collins, Ian Andrews, Kate McGill, Paul Hardy-Smith and Jun Hua Guo was formed. The steering committee members were invited to attend the steering committee meeting on 4 June 2019 at Rosehill Bowling Club. Four (4) steering committee members, one (1) NCCP representative, one (1) University of Canberra researcher, and two (2) FFVS staff members were in attendance to the meeting.

A biosecurity risk matrix was compiled to reflect potential risk pathways for CyHV-3. Biosecurity options were compiled from information gathered from the literature review performed in Phase 1 to mitigate the risk pathways identified in the matrix (see Appendix 4).

Koi farmers were contacted to discuss the feasibility and adoptability of biosecurity options. Only one (1) farm was available to comment. Subsequent attempts to contact additional farms were unsuccessful. Further input was sort after from Adrian Falconer as suggested by Jamie Allnutt. No response or input was received from Adrian Falconer.

Summary of the meeting and communications were detailed in Phase 3 (see Appendix 5, 6).

Phase 3:

An updated biosecurity options document was compiled prior to the second meeting held by teleconference on 25 June 2019 to consult with koi industry and Government on suitability and feasibility of biosecurity options.

Assessment of process of adoption

The biosecurity options document detailed in Phase 2 was updated with comments and suggestions by the steering committee members (see Appendix 5, 6). A teleconference was held on the 25 June 2019.

Meeting minutes were circulated to relevant people subsequent to the meeting (see Appendix 7). Additional comments were received by email from stakeholders who were unable to attend the conference. An additional attempt to contact farms was made by email and phone. Attempts were unsuccessful.

Phase 4:

Estimated costs associated with the feasible options were documented in Appendix 8 to develop biosecurity strategy for Australian koi industry in light of potential planned release of CyHV-3 including estimate of costs for Government and Industry.

Cost estimate of strategies to Industry and Government

Feasible options identified in previous stages of the project were identified and listed in a document (see Appendix 8). Factors influencing the adoptability of the feasible options were identified (see Appendix 5, 6, 7). Approximate estimate cost to implement feasible options were documented (see Appendix 8).

Phase 5:

Feasible biosecurity measures identified in previous stages of the project by stakeholders were summarised in Appendix 9 to develop biosecurity strategy for Australian koi industry in light of potential planned release of CyHV-3.

Recommendations of preferred biosecurity strategy and any associated work

A series of feasible biosecurity measures identified in previous stages of the project by stakeholders were compiled and detailed against their respective risk pathways (see Appendix 9). The risk to the koi industry and individuals with koi subsequent to applying the feasible biosecurity measures were detailed in the risk category section (see Appendix 9).

Additional research or project work required to underpin a strategy are outlined in the further areas of research and development section of the report (see Appendix 9).

Discussion and Conclusion

Through the process of consultation with stakeholders from industry it became apparent that not all of the biosecurity measures which had been identified from the literature review were considered practical and able to be implemented by the koi industry. The chosen measures were therefore not the most biosecure option identified by the literature review meaning that significant areas of risk are likely to remain.

Currently, industry wide adoption of quarantining is not possible. For many hobbyists, there is insufficient space and infrastructure to install a dedicated, biosecure, quarantine facility. The inadvertent introduction of latently infected, clinically normal koi into ponds/tanks remains a significant risk for the hobbyist industry, should CyHV-3 be released into open waters in Australia. Dedicated quarantine facilities were not available at the farm which participated in this project. Significant investment is required to erect dedicated quarantine facilities at koi farms.

No vaccinations for CyHV-3 are approved for use in Australia by the APVMA. Live attenuated vaccines have been used overseas and are reported to provide the highest level of protection. However, the use of live attenuated vaccines is not suggested in Australia due to the potential risk of spread of less virulent strains of CyHV-3 to wild carp populations thereby conflicting with the aim of the carp control program. Control over effluent discharge may not be possible for koi farms in all weather conditions. As such it remains plausible that a less virulent strain of the virus could be released into wild waterways. The stakeholders have highlighted that an efficacious vaccine would likely be used by hobbyists if available. The efficacy of killed vaccines was considered to be insufficient for the hobbyist industry to justify vaccinating their pet koi, even if they became commercially available. Further research and commercialisation of a more efficacious vaccine is suggested prior to the release of CyHV-3, if CyHV-3 is to be introduced into Australian waterways. By way of analogy, prior to release of calcivirus for rabbit control, an efficacious vaccine was available for hobbyist and commercial rabbit keepers.

Wildlife exclusion also poses a risk for the industry with the current proposed measures. Current use of bird nets is only limited to prevent bird predation when fish are smaller in both hobbyist and farm sectors. For koi hobbyist, bird nets are not always erected as it is visually unappealing, thus contrary to the reasons why hobbyist keep koi. In farms, the ongoing maintenance cost of erecting bird nets and the cost of the nets are uneconomic for farms. It has also been highlighted that exclusion of small birds is difficult for the farm. If CyHV-3 were to be released in Australia, further research into birds as potential vectors for CyHV-3 may assist in the understanding of this risk pathways for both farms and hobbyists.

Currently, both farmers and hobbyists have noted that disease investigations or disease screening (i.e PCR, histology, veterinary workup) are not adoptable for the industry due to factors including the diagnostic sensitivity and specificity of current tests for determining carrier fish status, cost of tests, and the availability and ease of access to diagnostic laboratories. Without disease screening and investigation, hobbyist and farmers can only speculate causes of disease events. Also, it has been highlighted by stakeholders that many koi hobbyist are unable to precisely recognise diseases or clinical signs of diseases. As such, there is significant risk of the disease disseminating to other populations unknowingly. The development of sampling methods which could determine sub-clinical carrier status with greater precision would be of value. The tests are already very sensitive at detecting the viral particles, however, in sampling fish non-lethally the presence of the virus can be missed.

The stakeholders have highlighted that non-koi club members will continue to pose significant risk to the koi industry as biosecurity information may not be readily available and easily distributed to said parties and uptake may be poor.

The likely efficacy of the combined biosecurity measures for an individual koi industry participant, who attends koi shows, or trades koi, is heavily contingent on a 100% uptake and full adoption of the biosecurity strategies. Such high levels of adoption in an industry with large numbers of hobbyists, is difficult/impossible to achieve in practice. Rigorous compliance monitoring may assist in improving uptake, however, would require significant recurring funding to maintain. Hence it will remain likely that some koi populations may become infected with CyHV-3 should the virus be released into Australia's wild waterways. Due to widespread fish movements within the koi industry, the inability for many koi keepers to quarantine koi, and the potential for movement of sub-clinical carriers, risk for unintended dissemination must be considered.

The greatest biosecurity protection that could be afforded to the koi industry is for the CyHV-3 release to not proceed, and for Australia to maintain its exotic disease status with respect to this pathogen.

Implications

The koi industry will now have access to assembled peer reviewed literature to inform their decisions around CyHV-3 and their farm, hobby and industry biosecurity practices. Improvements in biosecurity are anticipated as a consequence.

Government can be informed of the risks posed by a release of CyHV-3 to the koi industry. Government can use this project to assist in assessing the various proposed biosecurity measures within the Carp Control Program and their associated cost estimates.

Industry

The literature review in Phase 1 provide relevant details regarding CyHV-3 that is readily accessible, easy to read and compact for the koi industry. This document removes the need for koi keepers and farms from relying on grey literature which was found to be inconsistent and inaccurate in the literature review.

Should the CyHV-3 release proceed, these biosecurity measures will aid in providing increased biosecurity barriers for the koi industry against CyHV-3. Additionally, individual koi keepers and farms may also use the documents and previous biosecurity options detailed in Phase 1-3 of the project to identify areas where additional investment may facilitate in increasing biosecurity in ponds/ tanks/ farms.

General biosecurity practices suggested in this report may assist individual koi keepers and farms in preventing entry, spread and dissemination of diseases.

Additional research and education programs are likely to facilitate an improvement in biosecurity for koi keepers and farms against CyHV-3 and generally against other risks creating a lasting benefit.

Government

The findings and results of Phase 1-5 of project 2018-190 can assist government in the final assessment of the potential release of the exotic pathogen, CyHV-3. There are key intrinsic factors of the koi industry which suggest the development and adoption of additional biosecurity measures will be challenging. Should the koi industry adopt the many biosecurity measures outlined, then it is likely a demonstrable reduction in risk would be achieved at the individual hobbyist and farm level. However, the risks from CyHV-3 are unable to be reduced to zero within the constraints for adoption identified by industry. These factors are detailed in Phase 2 and Phase 3 of the project. It is clear from the literature review and consultation with the industry that CyHV-3 would pose a significant risk to the koi industry even if all the outlined feasible biosecurity measures are adopted.

Recommendations

Further research into CyHV-3 may offer opportunities for the industry to improve biosecurity against CyHV-3. Development of education programs will likely increase the biosecurity awareness of koi keepers and farmers.

Due to widespread fish movements within the koi industry, the inability for many koi keepers to quarantine koi, and the potential for movement of sub-clinical carriers, risk for unintended dissemination must be considered. To date, off-shore management in combination with on-arrival surveillance and quarantine of imported ornamental fish appears to have been sufficient to maintain Australia free of unplanned CyHV-3 introduction.

A recent study has highlighted the need to focus on off-shore management of diseases at the source, combined with on-arrival surveillance for preventing the risk of disease entry into Australia (Hood et al., 2019). An attempt to manage biosecurity risks for the koi industry in relation to CyHV-3 if released in Australia, would appear contrary to these acknowledged best practices of managing the risk off-shore. The strategy of managing disease risk from offshore has been a cornerstone of Australia's quarantine and biosecurity policy for many years, allowing Australian agribusiness to exploit its competitive production advantage and favourable market access due to freedom from many major international diseases.

The greatest biosecurity protection that could be afforded to the koi industry is for the CyHV-3 release to not proceed, and for Australia to maintain its exotic disease status with respect to this pathogen.

Further development

Further potential research projects:

- The effects of salinity on CyHV-3 infectivity and persistence in the environment.
- The efficacy of free chlorine at concentrations below 3mg/L on CyHV-3 infectivity at various virus concentrations for varying durations.
- The effects of ozone on CyHV-3 infectivity.
- The infectivity and viability of CyHV-3 from regurgitate/digested/faecal matter of birds that ingest infected carp/koi.
- Development of a sensitive and specific testing protocol to detect infected koi sick koi
- Development of a sensitive and specific testing protocol to detect latently, clinically normal infected koi.
- The infectivity (carrier/vector (mechanical and biological)) of artemia, daphnia, bloodworms to early life stages of koi.
- Development of a highly efficacious vaccine (~99%) that induces immunity for greater than one year against CyHV-3 that can be delivered by immersion bathe or oral ingestion.
- The efficacy and safety of Huwa-San© in a koi tank over a 24 hour period in preventing transmission of CyHV-3 to koi by cohabitation, aerosol, water transmission in transport stressed koi.
- Development of a pond side rapid detection test kit for latently infected, clinically normal koi.

Further potential education programs:

- Develop of education guideline for water quality, koi disease, sanitary practice
    - including risks of cohabitating other species with koi
    - including safe chemical handling and disinfection procedures
    - including safe chemical handling
    - including recognising signs of disease
    - including safe, sanitary disposal of effluent water
    - including safe mortality disposal practice

Extension and Adoption

Relevant industry bodies and government participants have been informed of outcomes of the project. This report is available publicly on FRDC. The Carp Control Program will be able to assist in dissemination of the report.

Project materials developed

See Appendix 3 – 9.

Appendices

Appendix 1 – List of researchers and project staff
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Appendix 3 – Phase 1 Literature review
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Appendix 1 – List of researchers and project staff

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Matthew A. Landos – Future Fisheries Veterinary Service – Principal Investigator

Tracey Kristiansen – Future Fisheries Veterinary Service – Administration/Finance

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Appendix 3 – Phase 1 Literature review

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Literature review of Biosecurity and Control strategies against Cyprinid Herpesvirus 3 (CyHV-3) in Australia in relation to the potential release of the virus as a biocontrol agent.

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Introduction

Cyprinid Herpesvirus 3 (CyHV-3) also known as Koi Herpesvirus (KHV) is a notifiable disease in koi (*Cyprinus carpio koi*) and European carp (*Cyprinus carpio*). CyHV-3 is the pathogen responsible for causing Koi Herpesvirus Disease (KHVD) which is the damaged tissues which result from viral replication. In subclinical infections with CyHV-3, no tissue damage may be evident, hence the fish is not technically diseased. It is however an infected carrier of the virus which could potentially spread to other susceptible fish. In recent years, CyHV-3 has been explored as a possible biocontrol measure for European carp in Australia. This virus has been detected in 33 countries including Canada, Indonesia, Japan, Taiwan, Thailand, Poland, Singapore, United Kingdom, and the United States of America.

The potential release of CyHV-3 into Australian waters would introduce new risks to Australia's koi industry. Mass mortalities of up to 100% have been described for captive carp infected with CyHV-3 (R. P. Hedrick et al., 2000). Due to the potential severity of risk outcomes the disease poses, it is important to understand the risk factors and potential biosecurity control measures available for the Australian koi industry.

This report reviews the different routes of entry of the virus into a koi population, and biosecurity and preventative measures described in published and grey literature. Biosecurity strategies employed by other countries are also explored.

Method

Research question

What is best practice for biosecurity strategies employed to control and/or prevent CyHV-3 in Koi fish?

Search protocol for peer reviewed journal

An online database search in Scopus, Web of Science, PubMed, Science Direct Freedom collection and general search with Google Scholar and OIE website were performed to answer the research question. The following criteria were used for each search:

- Must be an original research article.
- Full text must be available in English.
- Must be related to biosecurity, control and/or prevention.
- Must be published in a peer-reviewed journal
- Articles must be available online

The following search terms were used:

- Primary search terms: CyHV-3 OR Koi Herpesvirus
- *Subset search terms: Cyprinus carpio* OR Koi OR Carp
- Subset of subset search terms: Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*

All articles identified with the search protocol are complied. The titles of each search result were manually checked for relevance to the question. The abstracts of articles with relevant titles were checked for relevance to the question.

Grey literature

The following search criteria were used on 17/04/2019. Google and VIN were used to identify relevant grey literature articles. First twenty (20) webpage titles were scanned for relevance to the topic. Summary of relevant articles were presented in a table format.

- Full text must be available in English.
- Must be in text format
- Must be related to biosecurity, control and/or prevention.
- Articles must be available online
- Articles must not be published articles

Search terms:

- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3
- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 prevention
- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 disinfection
- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 vaccination
- Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 biosecurity

Review of previous Australian studies

Previous Australian studies were supplied by the NCCP. Other previous relevant Australian studies identified during the peer reviewed article search protocol were included. The strength of the evidence (strong, adequate, weak) and risk to various sectors of the Australian Koi industry are summarised in a table (see Grey literature, below).

Results

Relevant articles

Sixty-two (62) relevant research articles were identified with the search criteria (Appendix 1). Fourteen (14) relevant grey literature articles were identified with the search criteria.

Published literature

Database	Search terms	Number of results
Web of science on 14/03/2019	CyHV-3 OR Koi Herpesvirus	385
	Cyprinus carpio OR Koi OR Carp	381
	Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*	125
	Manual checking for relevance to topic	36

Table 6 Search results from the Web of Science on 14/03/2019.

Database	Search terms	Number of results
Scopus on 15/03/2019	CyHV-3 OR Koi Herpesvirus	298
	Cyprinus carpio OR Koi OR Carp	286
	Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*	285
	Manual checking for relevance to topic	39

Table 7 Search results from Scopus on 15/03/2019.

Database	Search terms	Number of results
PubMed on 19/03/2019	CyHV-3 OR Koi Herpesvirus	371
	Cyprinus carpio OR Koi OR Carp	359
	Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*	223
	Manual checking for relevance to topic	16

Table 8 Search results from PubMed on 19/03/2019.

Database	Search terms	Number of results
Science Direct on 26/03/2019	CyHV-3 OR Koi Herpesvirus	371
	Cyprinus carpio OR Koi OR Carp	359
	Outbreak* OR Mortalit* OR Prevent* OR Resist* OR Control* OR Detect* OR Case* OR Risk* OR Biosecurity OR Vaccin*	60
	Manual checking for relevance to topic	14

Table 9 Search results from Science Direct on 26/03/2019.

Grey literature

Search terms:

Search terms	Number of results
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3	14,300

Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 prevention	9,020
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 disinfection	6,000
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 vaccination	10,700
Koi herpesvirus Cyprinid herpesvirus 3 KHV CYHV 3 biosecurity	3,100

Table 10 Grey literature search results in Google.

Review of previous Australian studies

Four (4) articles were supplied by the NCCP. One (1) Australian peer-reviewed article, one (1) overseas case study, and two (2) grey literature articles were obtained.

One (1) additional previous Australian study was identified.

Discussion

Disinfection

UV

The use of disinfectants and UV light have been explored as potential control measures to prevent CyHV-3 entry to a facility. There are many different designs of commercial UV disinfection systems in aquatic systems. The ability of UV to kill a pathogen is measured in relation to the dose applied. This can be delivered through water, using external lamps. Commercial UV units are rated for the dose they deliver, relative to a flow rate moving through the unit. As the performance of UV units declines with the life of the bulb (speed varies between brands), it is recommended to install over-specified units that will deliver a protective UV dose, even at the end of bulb life.

A single peer-reviewed study was identified which demonstrated the efficacy of UV light, heat and disinfectants against CyHV-3 in petri dishes (Kasai et al., 2005). UV light was able to deactivate (100% plaque reduction) CyHV-3 (KHV-I strain) when exposed to doses at 4.0×10^3 $\mu Ws/cm^2$ (Kasai et al., 2005) using an external lamp directed at water containing the virus in a petri dish equivalent to 4 mJ/cm^2. These findings were higher than the dose rates effective in deactivating other herpesviruses such as Oncorhynchus masou virus (OMV) and similar structured (enveloped virus) viruses such as red sea bream iridovirus which require 1.0×10^3 $\mu Ws/cm^2$ to 3.0×10^3 $\mu Ws/cm^2$ (Kasai et al., 2002).

Another aquatic herpesvirus, ostreid herpesvirus 1 (OsHV-1), was found to be deactivated by UV treatment in highly filtered water. Unfiltered water may have higher levels of suspended solids, or other particulates, which can reduce the efficacy of UV disinfection as it allows potentially hazardous pathogens to be shaded from exposure to the UV light. If the UV beam does not directly hit the pathogen it will not be inactivated.

The dose rate required to deactivate pathogens can be affected by distance and exposure time. The required UV exposure dose was 15 mins at a proximity of 10cm to a UV lamp emitting a frequency of $1.08 mW/cm^2$ (Schikorski et al., 2011). Deactivation of OsHV-1 also occurs at higher UV doses of $>1000 mW/cm^2$ at 254nm at a distance of 15cm, for a duration of 10 minutes (Hick et al., 2016).

Chemical and thermal disinfectants

In addition to UV as a form of viral deactivation, varying concentrations of disinfectants including iodophors, sodium hypochlorite, benzalkonium chloride and ethyl alcohol were able to deactivate CyHV-3 at different temperatures (Figure 1) (Kasai et al., 2005). Chlorine concentrations of 0.3mg/L was able to deactivate 97.5% and 98.5% of the CyHV-3 when exposed for 20 seconds and 20 minutes respectively (Kasai et al., 2005). The authors recommended using 3mg/L of chlorine for deactivation of CyHV-3, however they did not specify a time period. FFVS suggest using the more conservative long exposure time of 30 minutes.

Disinfectant	Treatment time	Temperature (15°C)	Temperature (25°C)	Temperature (unspecified)
Iodophor (mg/L)	30 s	200	200	N/A
	20 min	200	200	N/A
Sodium hypochlorite solution (mg/L)	30 s	>400	>400	N/A
	20 min	200	250	N/A
Benzalkonium chloride solution (mg/L)	30 s	60	30	N/A
	20 min	60	30	N/A
Ethyl alcohol (%)	30 s	40	30	N/A
	20 min	30	25	N/A
Free Chlorine (mg/L)	30 min	N/A	N/A	3

Figure 1 Adopted table from Kasai et al., 2005 of the minimum concentration of disinfectants required for 100% plaque reduction at various temperatures (Kasai et al., 2005)

CyHV-3 was deactivated when exposed to temperatures greater than 50°C heat for more than 1 minute (Kasai et al., 2005).

Currently, there are no studies on the efficacy of ozone on CyHV-3, however the use of ozone has been explored with other herpesviruses in aquatic and terrestrial environments (Kasai et al., 2002; Petry et al., 2014). Kasai et al. demonstrated a >99% reduction in OMV viral infectivity when exposed to 0.5mg/L total residual oxidants (TRO) concentrations of ozone for 15 seconds (Kasai et al., 2002). Ozone concentrations of 0.02 to 0.05 mg/L were able to cause inhibition of 90% and 99.6% of HSV-1 and BoHV-1 (Herpes Simplex Virus 1 and Bovine Herpesvirus 1) respectively when exposed for 3 hours (Petry et al., 2014). More research would be required to define the efficacy of ozone on CyHV-3 infectivity.

Other studies investigating environmental disinfection have been found to be effective in preventing outbreaks and/or spread of CyHV-3 (Bergmann, Monro, et al., 2017). Huwa-San© (a hydrogen peroxide based disinfectant) was found to be effective in preventing the spread of CyHV-3 from sub-clinical CyHV-3 infected carp to naïve carp with a common water source in a recirculating system and also within the same tank through use of 60mg/L concentrations of Huwa-San© (Bergmann, Monro, et al., 2017).

A study in 2016 by Flamm et al. suggest that draining and liming of ponds (to achieve pH of up to 12) recently affected by CyHV-3 may potentially be effective in controlling the spread and prevalence of CyHV-3 in farmed carp when left without water to dry for a period of at least 6 weeks. Rates of lime required will vary with local water and soil conditions. For confidentiality reasons, Flamm et al. were not able to provide data surrounding the prevalence of CyHV-3 in untreated ponds (ponds that have not received liming and draining) but reported that the incidence rate of Koi Herpesvirus disease (KHVD) was lower in treated ponds (n=2) based on personal observations. However, the diagnostic tests used in the study pose potential reporting bias, as the author noted that the sensitivity and specificity of PCR tests used were, as yet, not validated.

Water, environment and predisposing factors for infection

Environmental factors can influence the infectivity of CyHV-3. The virus' infectivity was lost in natural waters at 15°C to 30°C in 3 days in the absence of a host (Shimizu et al., 2006; Yoshida et al., 2013). Temperatures outside this range were not examined. Bacteria may contribute to the inactivation of CyHV-3 in water (Shimizu et al., 2006; Yoshida et al., 2013). A variety of species of bacteria containing anti-CyHV-3 properties have been isolated from natural water and intestines of carp (Shimizu et al., 2006; Yoshida et al., 2013). This experiment took bacterial isolates from the intestine of fish and added them to cell cultures flasks containing CyHV-3 to determine if they reduced the impact of the virus on the cell line. So, the results are indicative only of in-vitro circumstances and have not been extrapolated into protection measures in practical koi holding facilities.

The infectivity of CyHV-3 was found to decrease with time during a seven day study (Shimizu et al., 2006). The infectivity and mortality rate of CyHV-3 decreased in common carp challenged by immersion with water that had been inoculated with virus prior to the challenge test (Perelberg et al., 2003). The three challenge groups were: immediate exposure post inoculation, 4 hours after inoculation, and 24 hours after inoculation (Perelberg et al., 2003). Significantly lower mortality rates were noted between the group challenged immediately after inoculation (approximately 90% mortality) compared to the other two groups (approximately 5% mortality) (Perelberg et al., 2003). In autoclaved or filtered water, the infectivity of the virus persisted for more than 7 days, suggesting bacteria were playing some role in accelerating degradation of the virus (Shimizu et al., 2006).

CyHV-3 DNA has also been detected in sediment samples, and may potentially be a reservoir for CyHV-3 (Honjo et al., 2012; Shimizu et al., 2006). However, the infectivity of the detected DNA was not tested in these studies (Honjo et al., 2012; Shimizu et al., 2006). Active CyHV-3 is shed in faeces of infected carp, and ingestion of infected material can results in the development of KHVD (Dishon et al., 2005; Fournier et al., 2012). While it is not known whether the virus detected in sediment samples are infective in vitro, the presence of detectable CyHV-3 DNA in sediment samples was found to move below detectable limits within 1-7 days at the permissive temperature range (15-30^{o}C only used in this study) in the absence of a host (Shimizu et al., 2006).

CyHV-3 DNA has been detected by PCR in pooled samples of zooplankton and phytoplankton, and pooled, grounded samples of mussels, and crustaceans (Kielpinski et al., 2010; Minamoto et al., 2011). Hence it is speculated that plankton, mussels and crustaceans may serve as a potential reservoir/vector for CyHV-3. Currently, there are no studies available on the infectivity/transmissibility of the detected virus to carp or koi through this vector.

Alterations to the body's immune function can increase susceptibility of fish to diseases (including CyHV-3). Stressful conditions such as transport, temperature change, handling, sampling, and breeding can result in lowered immunity and reactivation of CyHV-3 (Ashley, 2007; Eide et al., 2011; Lin et al., 2017). Organophosphates (OP) such as malathion have been demonstrated to decrease antibody production in carp vaccinated against *Aeromonas hydrophila* (Soltani et al., 2003). Removal/ damage to the skin's health and mucus may increase the susceptibility of koi and carp to CyHV-3 through compromised immunosuppression (Raj et al., 2011; Ronsmans et al., 2014). Carp larvae (6.9-8.7mm length) are believed to be more resistant to CyHV-3 due to differences in the innate immune system provided by the skin's mucus during early stages of development (Ito et al., 2007; Ronsmans et al., 2014). Carp larvae are significantly more susceptible to CyHV-3 when disruptions are made to the mucus layer and when challenged intraperitoneally with the virus rather than through challenge by bath immersion (Ronsmans et al., 2014).

Non-koi or carp hosts

Should koi keepers also keep other species, then the following risks should be considered.

CyHV-3 has been detected in many other non-koi and carp hosts. Although further work to validate non-target susceptibility is recommended (NCCP project 'Best practice in non-target species susceptibility testing'), potential for vectoring of CyHV-3 through non-target species should be considered. Viral replication has been detected in cell cultures of goldfish, silver carp and fathead minnow suggesting potential for susceptibility of non-koi or carp species to CyHV-3 (Davidovich et al., 2007). Goldfish, grass carp, blue black ide, and *Ancistrus sp.* potentially may be carriers of CyHV-3 (Bergmann et al., 2009). Carp and koi hybrids have been shown to be susceptible to CyHV-3 and the disease (Koi Herpesvirus disease), where tissue damage was evident (Bergmann et al., 2010; R. Hedrick et al., 2006). Goldfish appear to be unaffected by CyHV-3 and no viral DNA or clinical signs were detected (R. Hedrick et al., 2006). These findings differ from El-matbouli et al.'s findings in 2008 where CyHV-3 was detected in pooled samples of gills, kidneys, spleens and brains of 45 goldfish cohabitated with CyHV-3 infected koi (El-matbouli et al., 2008).

Stress appears to play a role in the susceptibility of target and non-target species to CyHV-3. Under stressful conditions (temperature stress), goldfish may potentially transmit CyHV-3 to naïve carp by cohabitation (El-Matbouli et al., 2011). No signs of the disease (Koi Herpesvirus disease), where tissues are damaged, developed in any of the goldfish. Topmouth gudgeon (*Pseudorasbora parva*) appears to only be susceptible to CyHV-3 when stress factors were applied (removal of skin mucus or scaring) (Pospichal et al., 2018).

Under experimental conditions, mortalities were observed in non-cyprinid species such as rainbow trout (*Oncorhynchus mykiss*), silver perch (*Bidyanus bidyanus*), Person's tree frog tadpole (*Litoria peronii*), and sea mullet (*Mugil cephalus*) when exposed to CyHV-3 by intraperitoneal injections (IP) and/or immersion bath (McColl et al., 2017). Although mortalities in the species described above were unexplained, it is important to note that no evidence of CyHV-3 was detected by RT-PCR. Also, no evidence of tissue damage associated with CyHV-3 were detected by histology. Silver perches and tilapia were found to be unaffected by CyHV-3 in a study in 2003 when cohabitated with CyHV-3 infected carp and no mortalities were observed (Perelberg et al., 2003). No histology or molecular testing was performed in Perelberg et al.'s study in 2003.

Further details are available within other NCCP projects.

Vaccination

The use of attenuated live vaccines, DNA vaccines, and liposome vaccines have been explored as potential control methods for CyHV-3 (Cui et al., 2015; Embregts et al., 2019; O'Connor et al., 2014; Perelberg et al., 2008; Yasumoto et al., 2006). There are many routes of administering these vaccinations including: immersion, injection (intra-muscular (IM), intra-peritoneal (IP)), and oral (PO) (Cui et al., 2015; Embregts et al., 2019; O'Connor et al., 2014; Perelberg et al., 2008; Yasumoto et al., 2006). Currently, no studies have demonstrated the repeatability of the vaccine trials, with studies examined here containing a single vaccinated and single control cohort. Greater confidence in vaccine performance could be achieved with repetition of these experiments at an additional research institute.

The efficacy of immersion baths varies considerably between studies for both DNA and live attenuated vaccines. The stocking density of fish within the immersion bathes appears to affect the survivability of juvenile common carp vaccinated against CyHV-3 by DNA vaccine. Survival rates of 31.2% and 82% were reported in fish vaccinated at 800 fish/L and 1200 fish/L immersion densities respectively (Aonullah et al., 2017).

Vaccination by immersion can be advantageous as it permits fish to be vaccinated in bulk numbers at any size in contrast to delivery by injection. Oral delivery of the vaccine coated on food is less effective in achieving significant immunostimulation (Embregts et al., 2019). Presently, there are no highly efficacious oral fish vaccines in the world. It remains an area of research interest.

Less pathogenic strains of CyHV-3, which lack virulence factors, have been used as live attenuated vaccines to prevent clinical infection (Boutier et al., 2015; Dishon et al., 2014; Nowak et al., 2006; O'Connor et al., 2014; Perelberg et al., 2008, 2005; Ronen et al., 2003; Schröder et al., 2019). These less pathogenic strains of CyHV-3 significantly reduce the severity of clinical disease observed with their infection. Vaccinated (infected with the live attenuated strain) fish become infected with the modified version of CyHV-3. Fish vaccinated with live attenuated vaccines will likely test positive for CyHV-3 by PCR due to the homology between the pathogenic CyHV-3 and the attenuated strain. It is unknown whether these less virulent strains of CyHV-3 can re-acquire/attain their removed/additional virulence factors and revert to their pathogenic state and cause clinical disease outbreaks (Hanley, 2011). Since the virus being used in these vaccine is alive, the risks for subsequent reversion to pathogenic strains remain, which is in contrast to DNA or killed adjuvanted vaccines which do not contain any live virus (Z. Liu et al., 2014). Live attenuated vaccines have been found to provide protection against CyHV-3 for up to 13 months, with survival rates of 64% (Connor et al., 2014). Antibody levels against CyHV-3 appear to peak around 3-4 weeks after vaccination, and gradually decrease with time (Perelberg et al., 2008). By day 280 post vaccination, antibody levels are marginally higher than that of unvaccinated fish (Perelberg et al., 2008). Horizontal transmission of the attenuated live strain of virus in the vaccine to naïve fish is known to occur (Weber III et al., 2014). Vaccinated fish were cohabitated with naïve fish 28 days post vaccination. Detectable levels CyHV-3 were detected by qPCR (Weber III et al., 2014). Temperature can greatly affect the efficacy of live attenuated vaccines (Perelberg et al., 2005). Survival rates of approximately 97% were seen in fish vaccinated at 23°C as opposed to fish vaccinated at 27°C which had survival rates of approximately 30% (Perelberg et al., 2005).

Specific vaccines

The efficacy of DNA intramuscular (IM) vaccine has been examined in several studies. A series of three (3) IM injections (injections performed every 3 weeks) with DNA (ORF25) vaccines have been found to provide protection against CyHV-3 (J.-X. Zhou et al., 2014). Survival rates between 80-87.5% were noted when fish were challenged by intra-peritoneal (IP) injections of CyHV-3. Similar findings were found by Liu et al. who reported 78.3% survival in fish vaccinated by IM injection and challenged with IP injections of CyHV-3 (L. Liu et al., 2018). Improved survival rates (86.7%) were noted when an adjuvant was added to the vaccine (L. Liu et al., 2018). Survival rates of fish vaccinated with a different encoding gene (ORF81) shared similar results to that of ORF25-based vaccine, with survival rates ranging between 82.5% to 85.0% (J. Zhou et al., 2014). Interestingly, survival rates were greatly diminished (less than 40% survival) when ORF25-based vaccinated fish were cohabitated with infected fish (Embregts et al., 2019). However, when challenged by immersion, 90% survival was achieved (Embregts et al., 2019). The authors recommended future trials use more natural exposure scenarios such as cohabitation to better assess vaccine efficacy.

The use of oral ORF25- based DNA vaccine was found to be ineffective against CyHV-3 (0% survival) (Embregts et al., 2019). Better success was found with ORF81-based oral DNA vaccines, with survival rates of 71% and 53% for carp and koi respectively (Cui et al., 2015). It is important to note that the carp in Cui et al.'s experiment were held at 15±1°C, which may potentially confound the results as the temperature falls around the minimum permissive temperature range for disease transmission. There is only evidence of transmission at or below this temperature in a single study (at 12-13 °C) (Baumer et al., 2013).

Anti-CyHV-3 IgY produced in chicken eggs when incorporated into diets appeared to provide some protection (50% survival) against CyHV-3 in carp when exposed to the lower dose rate of $40TCID_{50}$. The higher exposure cohorts had no improvement in survival after 28 days with only 15% surviving the challenge of $80TCID_{50}$. (Z. Liu et al., 2014). Immunoglobulin M (IgM- a type of fish antibody) levels of carp fed the anti-CyHV-3 IgY diet were reported to be elevated in survivors after the challenge trial. It is not known if the elevated IgM is as a consequence of the IgY administration in the feed, or as a consequence of infection during the challenge trial, as measurements were only undertaken after the challenge trial, and not prior. FFVS suggest that the constant long-term feeding of antibodies may not deliver long term protection due to immunoadaptation of the fish, and is likely to be impractical from a cost perspective. Liposomes (spherical vesicles used to deliver active materials (i.e. vaccine) to site of action) have also been used to deliver inactivated (dead virus) vaccines to juvenile carp through oral administration (Yasumoto et al., 2006). Survival rates of 76.7 – 76.9% have been reported in 30g juvenile carp administered liposome vaccines (Yasumoto et al., 2006).

Surveillance of disease

Surveillance is an important measure for identifying CyHV-3 infected fish. A recent study has highlighted the need to focus on off-shore management of diseases at the source, combined with on-arrival surveillance for preventing the risk of disease entry into Australia (Hood et al., 2019). The authors noted that historical reviews of the Australian biosecurity system did not fully manage the risks associated with ornamental fish importation. An attempt to manage biosecurity risks for the koi industry in relation to CyHV-3 if released in Australia, would appear contrary to these acknowledged best practices of managing the risk off-shore. The strategy of managing disease risk from offshore has been a cornerstone of Australia's quarantine and biosecurity policy for many years, allowing Australian agribusiness to exploit its competitive production advantage and favourable market access due to freedom from many major international diseases. To date, quarantine and ornamental fish import measures appear to have been sufficient to maintain Australia free of unplanned CyHV-3 introduction.

For diagnosis of the disease of CyHV-3, it is recommended by the OIE that a combination of two to three tests should be used in the diagnosis of CyHV-3 (4. Diagnostic methods) (OIE, 2018). PCR and ELISA tests are used commonly to identify CyHV-3 in koi and carp.

The use of ELISA (enzyme linked immunosorbent assay) tests has only been found to be effective for the determination of population disease status when used in sample populations of greater than ten fish, but is not sensitive or specific at an individual fish level (Bergmann, Wang, et al., 2017). There are various types of PCR tests available, including qPCR, RT-PCR (Bercovier et al., 2005; El-matbouli et al., 2007; Gilad et al., 2002, 2004; Gray et al., 2002; R. P. Hedrick et al., 2000; Yuasa et al., 2012, 2005). Samples for PCR testing can be obtained lethally and non-lethally (Bercovier et al., 2005; El-matbouli et al., 2007; Gilad et al., 2002, 2004; Gray et al., 2002; R. P. Hedrick et al., 2000; Monaghan et al., 2015; Yuasa et al., 2012, 2005). Non-lethal samples can be obtained from skin mucus, gill swabs and blood samples, and are reported to be more sensitive for CyHV-3 during early infection periods (less than 5 days post infection) (Monaghan et al., 2015). When sampling lethally (in organ tissues such as kidney, spleen and gill), qPCR appears to yield more diagnostic sensitivity and specificity compared to virus isolation and conventional PCR and may be suitable for disease surveillance programs (Clouthier et al., 2017). The precision of virus isolation results varied significantly between laboratories which was attributed to the different cell line culture's susceptibility to CyHV-3 and their stability (Clouthier et al., 2017). Cross contamination appeared to be more prevalent with the conventional PCR method which increased the number of false positive results (Clouthier et al., 2017). This issue with cross contamination was not seen any of the laboratories when using qPCR (Clouthier et al., 2017). qPCR has been selected as the diagnostic test of choice by Canada's NAAHP for testing apparently healthy or clinically diseased fish (Clouthier et al., 2017). Gilad et al.'s 2004 real time PCR is the preferred assay suggested by the OIE to diagnose DNA presence of CyHV-3 (OIE, 2018).

Latency of CyHV-3 poses a challenge for detecting virus infected fish. Latently infected fish appear clinically normal, but can shed virus and reactivate viral disease under stressful conditions (Bergmann et al., 2009; Lin et al., 2017). Detection of latently affected fish is complicated by the low numbers of viral genomes in latently infected cells. Latent CyHV-3 can be detected in less than 1% of peripheral white blood cells (B-cells) (Reed et al., 2014, 2015). A recombinase polymerase amplification (RPA) assay has been developed to detect latent infection in fish (Prescott et al., 2016). Twelve previously CyHV-3 exposed, non-clinical koi and one true negative koi were used during the study. Though there were low numbers of fish used during the study, 12/12 previously exposed fish were identified to be positive for CyHV-3 with the RPA assay compared to one fish identified as positive for CyHV-3 with qPCR. In clinically affected fish, an ELISA has been developed for pond side detection of diseased koi. However, the sample size of the experiment was small (n=6) and diagnostic sensitivity of the test is relatively poor compared to PCR tests (52.6% relative to Bercovier et al. 2005's PCR protocol) (Vrancken et al., 2013)

Placing fish under stress is a method used to attempt to increase the expression of any latent pathogen, such that it becomes easier to detect. Imparting stressors to aid identification of latently infected fish have also been explored (Eide et al., 2011; Lin et al., 2017). Transport stress was used in Huchzermeyer et al.'s report in 2015 as part of a quarantine and active surveillance program to screen for CyHV-3 in a subpopulation of koi quarantined for 14 days at a permissive temperature (Huchzermeyer et al., 2015). Approximately 400-800 fish from the farm were sampled every 6 months, and 150 fish were sacrificed for PCR testing performed on gill swabs and viral cultures performed on internal organs – all results were negative for CyHV-3. Other potential stress factors such as temperature, handling, and sampling stress can potentially increase the number of CyHV-3 genomes detectable by diagnostic tests (Eide et al., 2011; Lin et al., 2017).

Foreign countries' approach to CyHV-3 management

FFVS selected some foreign countries for further investigation of their biosecurity measures with respect to CyHV-3. As the release of CyHV-3 is being considered as a potential biocontrol agent in Australia to control wild European carp populations, countries that are free from CyHV-3 were excluded. Non-English speaking countries or countries with published articles unavailable in English were excluded. The United Kingdom, South Africa, Canada, Singapore and Japan were selected as countries of interest as published case reports of CyHV-3 outbreaks or control/management strategies of CyHV-3 were available online. Officials in South Africa and Canada did not respond to multiple requests for assistance.

United Kingdom Government

CyHV-3 is a notifiable disease under The Aquatic Animal Health (England and Wales) Regulation 2009 in the UK (Cefas, 2019; Legislation UK, 2009). The virus is present widely throughout the country, and antibodies are readily detectable in carp throughout England's and Wales' fisheries (Taylor, Dixon, et al., 2010). It is speculated that live fish movement was responsible for the spread of the virus in the UK (Taylor, Way, et al., 2010).

The UK currently does not perform active surveillance of CyHV-3, and there are no attempts to eradicate the disease due to the technical difficulties and economic cost implicit in the process.

Outbreaks of CyHV-3 occur most commonly in recreational stillwater carp fisheries (used for angling) (Edmund Peeler pers. comm,. 2019). Low prevalence of the virus has been detected in some farms in the UK (Edmund Peeler pers. comm., 2019). The UK's policies aim to prevent the spread of the virus during outbreak scenarios due to the difficult nature of removing fish from a semi-wild environment (e.g. lake systems). The following measures are used during CyHV-3 outbreaks in recreational stillwater carp fisheries:

- Additional biosecurity communication placed around the fishery (signs, footbath stations, others)
- Fish movement restriction for a minimum of one year (or until water temperatures fall below permissive temperatures the following year).
    - Additional restrictions are placed on introduction of new fish into the fisheries during this period
    - Angling is prohibited during this period

For ornamental fish, there is a general reliance on importer's health certifications to exclude exotic diseases. As CyHV-3 is not an exotic disease in the UK, CyHV-3 is not included in the health certification for required testing on entry. As for the recreational sector, no active surveillance is employed to detect CyHV-3. For some exotic disease (e.g. Spring viraemia of carp), the UK does undertake active surveillance for susceptible species (Edmund Peeler pers. comm., 2019). The response to CyHV-3 outbreaks differ in the ornamental sector compared to the recreational sector, with all fish culled on site (Edmund Peeler pers. comm.,2019). No compensation is awarded to either the recreational nor ornamental sector in the event of compulsory culling.

Singapore Government

The first outbreak of CyHV-3 was detected in Singapore in 2006. Ling et al. (2005) published an article detailing the quarantine, surveillance and monitoring methods used in Singapore during 2005 (Ling et al., 2005). The quarantine protocol used during that period was as follows:

- Quarantine of all koi from Japan and Indonesia for a minimum of three (3) weeks.
- Sampling of koi or sentinel koi (sentinel koi are CyHV-3 free stock considered of low value, such that losses from infection in a cohabitation trial are considered economically tolerable) cohabitated with imported koi for 7 days for tissue culture (and PCR (Susan Kueh pers. comm., 2019))
- Quarantine koi are only discharged from the isolated quarantine centre after test results are obtained from the laboratory and if negative for CyHV-3
- All quarantine water is disinfected with chlorine prior to discharge into the sewage system

Since 2009, amendments to the testing protocols have been made in Singapore. Real-time PCR (Gilad et al., 2004) have been used since 2009 (Diana Chee pers. comm., 2019). For valuable koi, 30 sentinel koi are cohabitated with the valuable koi and samples are obtained from the 30 sentinel koi and tested by the methods described in Gilad et al. 2004 (Diana Chee pers. comm., 2019).

Japan Government

PCR testing and biosecurity measures are implemented at the Niigata prefecture of Japan to prevent the introduction and spread of CyHV-3 into koi populations. Only less expensive young (less than one year old) koi (sentinel koi) are destructively sampled for PCR. These sentinel koi are cohabitated with more expensive fish destined for commercial use and held at 20ºC to 25ºC for 3 weeks (Yamada et al., 2005). Deliberate stressors are not used to challenge these fish. No positive fish were detected in the 2005 report; however, farmers were required to report any suspected CyHV-3 outbreaks to the government. Upon confirmation of the disease, transportation of any fish from the infected site was prohibited, and all fish were incinerated or buried (Yuasa et al., 2009).

All fish shipped from another breeder within Japan, were required to be quarantined separately from existing stock and held at a low temperature (unspecified in article) in tanks for three weeks in accordance with policy (Yamada et al., 2005). Staff members are required to monitor and record the health of new fish during this period.

Breeders are advised to avoid using river water to perform water exchanges, and to sanitise and clean equipment that has been in contact with water or fish (Yamada et al., 2005)(Yamada et al., 2005)(Yamada et al., 2005)(Yamada et al., 2005)(Yamada et al., 2005) Personal biosecurity is recommended, and staff are required to wash their hands and disinfect footwear prior to entry to any facilities. Fish health records are required to be recorded daily (Yamada et al., 2005).

Grey literature

It is the view of FFVS that many of the conclusions reached within grey literature sources are not supported by strong verifiable evidence. As such, all grey literature conclusions must be viewed with considerable caution, as they may represent opinion rather than scientific evidence. Hence it is risky to consider using grey literature recommendations as the basis for biosecurity strategies for the koi industry.

Article	Topic	Information	References
1	Vaccination	• Vaccinations are unlikely to last more than 6 months. • There are potential risks of the vaccine reverting to pathogenic viruses	(Practical Fishkeeping, 2016)
	Testing	• PCR can be used to diagnose CyHV-3 • Faeces can be used to detect virus and antibodies in KHVD survivors • Faeces remain infective even at non-permissive temperatures	(Practical Fishkeeping, 2016)
	Quarantine and biosecurity	• Quarantine in separate system • Dedicated quarantine equipment in quarantine area • Quarantine period: 2 weeks at 25ºC	(Practical Fishkeeping, 2016)
	Transmission	• Fish transport • Illegal trade • Through contaminated water and fomites	(Practical Fishkeeping, 2016)
	Susceptible species	• Koi and carp • Flathead minnow • Silver carp, goldfish	(Practical Fishkeeping, 2016)
	Latency	• Fish held at low temperatures may mask disease • Disease only manifests between 23ºC and 28ºC	(Practical Fishkeeping, 2016)
2	Transmission	• Contact (infected fish, water, mud, fomites)	(Towers, 2013)

	• Disease transmission through gills and skin • Infection is temperature dependant (only occurs between 15.5°C to 28 °C	
Latency	• Infected fish may become carriers of the virus • As infected fish may become carrier, depopulation is recommended	(Towers, 2013)
Vaccination	• Vaccinated fish may develop antibodies against CyHV-3 • Length of protection against CyHV-3 is unknown • Live vaccine available for fish above 100g, however, the efficacy and duration of protection provided is debatable. • Diagnostic tests cannot differentiate vaccine strains and wild strains of CyHV-3.	(Towers, 2013)
Testing	• Cell cultures • PCR • Samples can be lethal and non-lethal • ELISA test • Negative results do not indicate that the fish is not a carrier of the disease	(Towers, 2013)
Disinfection/inactivation	• All materials in contact with infected water should be disinfected • Virus in water without a host may persist for up to 3 days. • Equipment should be cleaned before disinfection with: • Chlorine at 200ppm (200mg/L) for one hour or; • Bleach (5.25% Sodium hypochlorite) at 200ppm (200mg/L) • Quaternary ammonium compound (QAC) at 500ppm (500mg/L) for one hour – rinse thoroughly after use	(Towers, 2013)
Quarantine/Biosecurity	• New fish are quarantined in a different system that is in a different building or area to resident fish for a minimum for 30 days. • Separate equipment dedicated for quarantine fish is required • Footbath and hand sanitisers used upon entry and exit of the quarantine area • Sick fish should be examined by a veterinarian • Blood samples should be collected from all healthy fish and submitted for ELISA • English-style koi shows should be used to prevent risk of cohabitation with infected fish • Fish returning from shows should be quarantined for 30 days at 24°C • On completion of the quarantine period, few new fish and few old fish should be cohabitated	(Towers, 2013)

		separately as part of a challenge test	
3	Treatment	1. Isolate infected fish from other fish 2. Increase temperature slowly to 86°F (30°C) by 2°F (1.1°C) per hour 3. Maintain temperature at 86°F (30°C) for 7 days and stop feeding 4. Reduce temperature to 80°F (26.7°C) after the treatment 5. Start Koi Fix for Food or Koi Fix for Water and Forma-Green (all three products are of unknown chemical(s), concentration(s), form(s), but make mention that they are antibiotics) and treat for 2-3 weeks 6. Treatment is effective and virus are dying if the mucus changes from yellow/green colour to beige	(National Fish Pharmaceuticals, n.d.)
	Latency	• KHVD survival fish do not become latent carriers of CyHV-3.	(National Fish Pharmaceuticals, n.d.)
4	Quarantine/Biosecurity	• Fish must be quarantined at temperatures above 22°C for at least 3 weeks • During the quarantine period, cohabitate one 'old' fish with the 'new' fish to check for latently infected fish	(White et al., n.d.)
	Other host species	• Survival rate of goldfish and tench to CyHV-3 is high and survivors may become carriers of the virus.	(White et al., n.d.)
5	Treatment	• None • Recovered fish become carriers of the virus • Fry are more susceptible to CyHV-3 than adults	(Avian and Exotics Animal Care Veterinary Hospital, n.d.)
6	Testing	• PCR on moribund sick fish – best results • ELISA test – can test for exposure to CyHV-3 non-lethally	(Healthy Koi, n.d.)
	Transmission	• Fish can become infected with CyHV-3 when they are exposed to or in direct contact with infected water; infected fish; infected effluent • Transmission from non-koi or carp cyprinid species does not occur • Recovered fish become latency carriers of the virus	(Healthy Koi, n.d.)
	Outbreak options	• Cull all stock • Give stock away to person with a CyHV-3 endemic ponds • Keep stock	(Healthy Koi, n.d.)
7	Quarantine/Biosecurity	• Quarantine fish for an extended period at 87°F (30.6°C) with 3-6 ppt (3-6 g/L) salt and praziquantel • Quarantined animals/survivors may be carriers of CyHV-3 • Quarantined fish are biopsied	(Johnson, 2018)
8	Testing	• Virus isolation • Histology • Microscopic pathology (TEM) • PCR • ELISA	(Waltzek, 2015)
	Quarantine/Biosecurity	• Good biosecurity practices	(Waltzek, 2015)

		• Only stocking fish that have disease freedom status and have had proper diagnostic testing performed • Vaccination may have questionable efficacy	
9	Quarantine/Biosecurity	• 4-6 week minimum quarantine period • Fish quarantined in a separate system, with separate equipment preferably as far away from the main system as possible. • Fish are quarantined at 60-77^{o}F (15-25^{o}C)	(Aquatic Veterinary Services, n.d.)
	Transmission	• Direct contact with infected fish, water, or equipment	(Aquatic Veterinary Services, n.d.)
	Testing	• PCR for active infections on live or dead fish – testing should not be performed on non-clinical fish • ELISA for non-clinical fish	(Aquatic Veterinary Services, n.d.)
10	Quarantine/Biosecurity	• Leave nets out in sun to dry • If no space is available, leave nets out in sun for 45 minutes before use • Dispose of mortalities properly and ensure no effluent water enters the system • Sterilise nets with chemicals (own and visitors')	(AnglingTimes, 2016)
11	Quarantine/Biosecurity	• Following a CyHV-3 outbreak, all fish were culled, and ponds were sanitised • New fish are quarantined for 30 days in a separate, isolated facility. • Fish from each different breeder are housed in separate tanks. • In house PCR and external reference laboratories used to test for CyHV-3	(Kodama Koi Farm, 2019)
12	Disinfection	• Virkon S (5g/1000L) can be used to disinfect ponds • Sandblasting and recoating of ponds alone is sufficient in disinfecting ponds. • Hold water for 12 hours in absence of a host to kill CyHV-3 virus • High dose of chlorine (unspecified concentration) • Potassium permanganate (1kg into pond) can be used to disinfect ponds	(Koi4U, 2011)
	Other host species	• Bacteria	(Koi4U, 2011)
13	Disinfection	• 4mJ/cm^2 of UV is required to deactivate CyHV-3	(Ultimate Reef, 2016)
14	Transmission	• Contact with infected fish, fluid, water, and mud of infected systems • Fomites, birds, aquatic mammals	(Broughon, n.d.)
	Quarantine/Biosecurity	• Stock fish of known origin, health status, health history and age • Equipment such as nets should be dried for 48 hours in the sun prior to use and/or disinfected with	(Broughon, n.d.)

	disinfectants such as: Virkon S; iodine-based disinfectants.

Table 11 Summary of grey literature articles

Previous Australian studies

Title	Type	Summary	Strength of evidence	Reference
Characteristics of cyprinid herpesvirus 3 in different phases of infection: Implications for disease transmission and control	Peer-reviewed study **(strong)**	Recently CyHV-3 infected fish may not develop clinical signs of KHVD if held at non-permissible temperatures.	**Strong**	(Sunarto et al., 2014)
		When temperatures are permissible, clinical disease and transmission can occur.	**Adequate** – fish were held at non-permissible temperatures for approximately 23 days. In normal river environments, such as the Yarrawonga Weir, temperatures may be non-permissive for CyHV-3 for approximately 150 days (MDBA, 2019).	
		Detection of CyHV-3 in persistently infected fish (fish held at non-permissible temperatures) may be difficult due to the low level of gene expression expressed.	**Strong**	
Cyprinid herpesvirus 3 as a potential biological control agent for carp (Cyprinus carpio) in Australia: susceptibility of non-target species	Peer-reviewed study **(strong)**	Non-cyprinid species are not susceptible to CyHV-3.	**Weak** – no investigation into the cause of mortality for animal groups (rainbow trout (*Oncorhynchus mykiss*), silver perch (*Bidyanus bidyanus*), Person's tree frog tadpole (*Litoria peronii*), and sea mullet (*Mugil cephalus*)) with high percentages of mortalities when exposed to CyHV-3 by IP injections and/or immersion bath	(McColl et al., 2017)
Production of Koi Herpesvirus-Free Fish: Implementing Biosecurity Practices on a Working Koi Farm in South Africa	Published case study **(adequate)**	Destruction of all fish on site except for broodstock that were not exposed to CyHV-3	**N/A** – there is an inherent risk that the broodstock may be latently infected fish which may be a source of persistent infections and disease transmission.	(Huchzermeyer et al., 2015)
		Hatchery and grow-out ponds were disinfected	**N/A** – other disinfectants such as iodophor and ethyl	

		with chlorine and dried for several weeks.	alcohol can be considered.	
		Water access for each biosecurity zone is separate from each other.	**N/A**	
		All potentially infected fish are destroyed.	**N/A**	
		Wild fauna (e.g. birds, fish) access to ponds are restricted.	**N/A**	
		Veterinary inspection and sampling were performed every 6 months.	**N/A**	
		Every 6 month, 400-800 fish from the cull group were selected and subjected to transport stress.	**N/A**	
		Fish were quarantined in a laboratory for 14 days. Gill swab samples were obtained for PCR and organ samples were collected for virus culture		
		Disinfection of hatchery ponds routinely performed with iodophores between hatchery runs.	**N/A**	
		Grow-out ponds operated on an all in all out system.	**N/A**	
		Ponds are dried after each grow-out period	**N/A**	
KHV (CyHV-3) and the Case for Biosecurity in the Koi and Carp Industries	Grey literature **(weak)**	Vaccinations have limited use in preventing CyHV-3.	**Weak** – no in line referencing for some statements given. Some statements made to reach conclusion conflict with later statements made. Some statements are made based on personal observations.	(Benjamin, 2008)
		Vaccination, sanitation, culling, movement restriction, fauna control, pond management are potential control strategies against CyHV-3	**Adequate**	
		Naïve fish are routinely introduced into outdoor ponds for	**Weak** – No diagnostic procedures are used	

		extended periods as a part of a challenge test to test for CyHV-3 presence. Absence of disease is correlated with absence of CyHV-3.	to confirm whether CyHV-3 is present. No articles cited to support challenge test procedure.	
		Possible entry pathways of CyHV-3 include: 1. Incoming and outgoing water 2. Introduced fish and ova onto farm 3. Feed 4. People and fomites 5. Wild fauna 6. Dead fish	**Adequate**	
		Disease prevention is easier to manage off site.	**Adequate**	
Biosecurity and the Ornamental Fish Industry **"Future proofing the industry"**	Grey literature **(weak)**	Any fish displaying signs of disease during quarantine should be tested.	**N/A**	(OATA, 2012)
		Quarantine duration should be at least 2 weeks and at 23-28°C.	**N/A**	
		Water should be discharged into sewage and not into natural or open water bodies.	**N/A**	
		Fish can be stressed to induce clinical disease	**N/A**	

Table 12 Summary risk and strength of evidence of articles provided by the NCCP.

Best practice biosecurity options

The following best practice biosecurity options have been compiled by FFVS from literature search results and from communications with government officials.

Pathogen entry

Water

Hold all incoming surface water which has not been disinfected for seven days (note that town water is considered to be disinfected water, due to chlorine use, and bore water is considered to be free from fish pathogens) in a fish-free tank. Additional water security can be gained by then disinfecting water with disinfectants such as iodophors, sodium hypochlorite, benzalkonium chloride and ethyl alcohol at doses stated in Figure 1, or alternatively use UV disinfection at a dose of 4.0×10^3 $\mu Ws/cm^2$. For koi shows, an additional disinfection option with a hydrogen peroxide based solution (i.e. Huwa-San©) at 60mg/L can be added into tanks to reduce risk of disease transmission through aerosol movement, water movement and contact with infected koi. The disease status of fish at a koi show is unknown and sublethal carriage of CyHV-3 has been documented. Hence the use of peroxide in show tanks can help minimise disease transfer risk during these events. Caution is required in higher water temperatures (>20°C) if holding for longer than one hour as gill toxicity can occur. Where possible, source sanitary water for each system to reduce risk of spreading disease in contaminated water between systems. Avoid tank effluent water being re-used across multiple systems.

Non-koi or carp species

Do not cohabitate koi or carp with goldfish, silver carp, fathead minnow, grass carp, blue black ide, *Ancistrus sp.*, koi/carp hybrids, Topmouth gudgeon (*Pseudorasbora parva*) which been reported to potentially harbour the virus. Additional species may be identified following further non-target species (NTS) testing as recommended by the NCCP report.

People and fomites

People and fomites are recognised as potential vectors for spread of aquatic viruses. Hence on first principles it is prudent to disinfect all equipment with chemicals at doses stated in Figure 1 after each use. Avoid using equipment foreign to systems, but rather use dedicated equipment for each system. If foreign equipment must be brought into a system, then it must be disinfected prior to use. Disinfect hands and footwear upon entry to koi systems. Discourage visitors from touching the tank walls, water or fish.

New fish

Quarantine all new fish at permissive temperatures for three weeks. Cohabitate new fish with thirty low value CyHV-3 free sentinel koi fish. Stress the new koi fish with transport, temperature, handling, and/or with CyHV-3 sampling. Cull all sentinel fish at the end of the quarantine period and perform at least two (2) to three (3) diagnostics tests (real time PCR, nested PCR, qPCR) and histopathology. New fish are only to be introduced into the system once diagnostic test results have been received and are negative for CyHV-3.

Other fauna

Avoid introducing fauna, particularly plankton, molluscs and crustaceans from infected waters into koi populations. Erect bird netting to prevent bird access to koi populations.

Pathogen spread within facility

Vaccination

Koi operators should understand that no vaccines are currently registered in Australia for control of CyHV-3 and that the process of registration takes several years. Further, peer reviewed articles suggest that clinical trials of live attenuated vaccines are unlikely to provide higher than ~80% protection. The use of attenuated live vaccines is not recommended in Australia due to the potential risk of spread of less virulent strains of CyHV-3 to wild carp populations. Should wild carp become immune to CyHV-3 it will adversely affect the killing efficacy of the use of CyHV-3 as a biocontrol agent in Australia. Alternative vaccines which have been described offer lower levels of protection from approximately 40-70%. Options include: oral DNA vaccines (ORF81) and liposome vaccines. Oral IgY may provide some protection against CyHV-3, during and outside of an outbreak, however it is not commercially available at this time in Australia.

Once fish are within a CyHV-3 free koi facility, avoid stressing fish where possible by avoiding temperature stress, transport stress, handling stress, breeding stress where possible. Additionally, avoid the use of organophosphate insecticides and/or water potentially contaminated with organophosphates when vaccinating fish as it may impair the generation of immunity. Seek advice from a veterinarian with expertise in koi disease prior to attempting to treat parasitic infections. General avoidance of pesticide exposure for stress reduction is recommended.

Existing stock

In the event of a fish getting sick in a koi population, undertake veterinary outbreak investigation, including the testing for presence of CyHV-3 using at least two to three diagnostic tests (real time PCR, nested PCR, qPCR) with histology, on appropriately selected, typically affected sick fish by attending veterinarian. In the event of a confirmed outbreak, cull all fish, hydrated lime (CaOH), and dry ponds in sunlight for minimum 7 days. Cease movement of all fish and equipment off site. Disinfect all effluent water prior to discharge (Figure 1). Increase biosecurity measures on site (i.e. foot bath and hand wash/sanitisation stations, biosecurity signage) to contain the outbreak to the one site.

Pathogen spread out of facility

Water

Disinfect all effluent water with chemicals detailed in Figure 1 and/or hold effluent water for seven days without a natural host prior to discharge.

Animals

Ensure that bird nets are erected and secure to prevent bird access to pond water. Install bird scaring devices to reduce risk of wild birds potentially regurgitating potentially infective material into the koi site. Discard all mortalities and euthanised sick animals appropriately into sealed plastic bag to land-fill or deep (>1m) onsite burial to avoid carcasses being dug up by vermin. Never release koi into waterways.

Cautionary statement

It should be noted that there are presently no DNA vaccines approved for use in fish in Australia by the Australian Pesticides and Veterinary Medicines Authority. Further, there are no vaccines of any kind approved for control of CyHV-3 in Australia. The process of regulatory approval requires assessment of a significant dossier of data and typically takes more than 2-3 years to gather data to submit for assessment. A single supportive study is insufficient. Should release of CyHV-3 be approved as a biocontrol measure in Australia, it is recommended that this process be undertaken to ensure availability of effective vaccines as quickly as possible to avoid risk to koi.

The likely efficacy of the combined biosecurity measures for an individual koi industry participant, who attends koi shows, or trades koi, is heavily contingent on a 100% uptake and full adoption of the biosecurity strategies above. Such high levels of adoption in an industry with large numbers of hobbyists, is difficult/impossible to achieve in practice. Rigorous compliance monitoring may assist in improving uptake, however, would require significant recurring funding to maintain. Hence it will remain likely that some koi populations may become infected with CyHV-3 should the virus be released into Australia's wild waterways. Due to widespread fish movements within the koi industry, and the potential for movement of sub-clinical carriers, risk for unintended dissemination must be considered. The international case study literature indicates that even with different levels of biosecurity in place, occasional outbreaks of CyHV-3 among commercial fish stocks continue to occur. In these events, some stock is invariably lost, as none of the currently available vaccines demonstrate 100% efficacy.

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Conflict of interest

None.

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Appendix 1 Database search results

Title	Scorpus	PubMed	Web of Science	Science direct	Replicates
A model to approximate lake temperature from gridded daily air temperature records and its application in risk assessment for the establishment of fish diseases in the UK	0	1	0	0	0
Analysis of stress factors associated with KHV reactivation and pathological effects from KHV reactivation	1	0	0	1	1
Antibody response and resistance of Cyprinus carpio immunized with cyprinid herpes virus 3 (CyHV-3)	1	1	1	1	3
Antiviral activities of Clinacanthus nutans (Burm.f.) Lindau extract against Cyprinid herpesvirus 3 in koi (Cyprinus carpio koi)	1	0	1	0	1
Can water disinfection prevent the transmission of infectious koi herpesvirus to naïve carp? – a case report	1	1	1	0	2
Characteristics of cyprinid herpesvirus 3 in different phases of infection: Implications for disease transmission and control	1	0	1	1	2
Comparison of the resistance of selected families of common carp, Cyprinus carpio L., to koi herpesvirus: Preliminary study	1	0	0	0	0
Construction of KHV-CJ ORF25 DNA vaccine and immune challenge test	0	0	1	0	0
CYHV-3 INFECTION DYNAMICS IN COMMON CARP (CYPRINUS CARPIO) - EVALUATION OF DIAGNOSTIC METHODS	0	0	1	0	0
Cyprinid viral diseases and vaccine development	1	0	1	1	2
Detection and significance of koi herpesvirus (KHV) in freshwater environments	1	0	0	0	0
Detection of cyprinid herpesvirus type 3 in goldfish cohabiting with CyHV-3-infected koi carp (Cyprinus carpio koi)	1	0	0	0	0
Detection of cyprinid herpesvirus-3 DNA in lake plankton	1	0	0	0	0
Disrupting seasonality to control disease outbreaks: the case of koi herpes virus	0	1	0	0	0
Do wild fish species contribute to the transmission of koi herpesvirus to carp in hatchery ponds?	1	0	0	0	0

Draining and liming of ponds as an effective measure for containment of CyHV-3 in carp farms	1	1	0	0	1
Effect of water temperature on mortality and virus shedding in carp experimentally infected with koi herpesvirus	1	0	0	0	0
Effects of daily temperature fluctuation on the survival of carp infected with Cyprinid herpesvirus 3	1	0	1	1	2
Effects of temperature on culture in vitro and pathogenicity of Cyprinid herpesvirus 3 [温度对锦鲤疱疹病毒体外培养和致病性的影响]	1	0	0	0	0
Efficacy and safety of a modified-live cyprinid herpesvirus 3 vaccine in koi (Cyprinus carpio koi) for prevention of koi herpesvirus disease	1	1	1	0	2
Efficacy of koi herpesvirus DNA vaccine administration by immersion method on Cyprinus carpio field scale culture	1	0	1	0	1
Feeding Cyprinus carpio with infectious materials mediates cyprinid herpesvirus 3 entry through infection of pharyngeal periodontal mucosa	1	0	0	0	0
Generation and characterization of koi herpesvirus recombinants lacking viral enzymes of nucleotide metabolism	0		1	0	0
Generation of a potential koi herpesvirus live vaccine by simultaneous deletion of the viral thymidine kinase and dUTPase genes.	0	1	0	0	0
Genetic analysis of common carp (Cyprinus carpio) strains. II: Resistance to koi herpesvirus and Aeromonas hydrophila and their relationship with pond survival	0	0	1	0	0
Inactivation of koi-herpesvirus in water using bacteria isolated from carp intestines and carp habitats	1	1	1	0	2
Intra-muscular and oral vaccination using a Koi Herpesvirus ORF25 DNA vaccine does not confer protection in common carp (Cyprinus carpio L.)	1	1	1	1	3
Koi herpesvirus: distribution and prospects for control in England and Wales	1	1	1	0	2

Koi herpesvirus: Status of outbreaks, diagnosis, surveillance, and research	1	0	0	0	0
Managing the koi herpesvirus disease outbreak in Indonesia and the lessons learned	1		0	0	0
Melissa officinalis L. extract and its main phenolic compound rosmarinic acid as phytoprophylactic feed additives against koi herpesvirus infection in a pilot study	1	0	1	0	1
Modelling the koi herpesvirus (KHV) epidemic highlights the importance of active surveillance within a national control policy	1	0	0	0	0
Molecular comparison of isolates of an emerging fish pathogen, koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi	1	0	0	0	0
Oral immunization of common carp with a liposome vaccine fusing koi herpesvirus antigen	1	0	1	0	1
Oral passive immunization of carp cyprinus carpio with anti-CyHV-3 chicken egg yolk immunoglobulin (IgY)	1	0	1	0	1
Preparation of monoclonal antibodies against KHV and establishment of an antigen sandwich ELISA for KHV detection	1	1	1	1	3
Production of Koi Herpesvirus-Free Fish: Implementing Biosecurity Practices on a Working Koi Farm in South Africa	1	0	0	0	0
Production of monoclonal antibody against ORF72 of koi herpesvirus isolated in Taiwan	1	1	1	0	2
Protective immunity of a modified-live cyprinid herpesvirus 3 vaccine in koi (Cyprinus carpio koi) 13 months after vaccination	1	1	1	0	2
Rational development of an attenuated recombinant cyprinid herpesvirus 3 vaccine using prokaryotic mutagenesis and in vivo bioluminescent imaging.	0	1	1	0	1
Recombinant lactobacillus expressing G protein of spring viremia of carp virus (SVCV) combined with ORF81 protein of koi herpesvirus (KHV): A promising way to induce protective immunity against SVCV and KHV infection in cyprinid fish via oral vaccination	1	1	1	1	3
Reservoirs of Cyprinid herpesvirus 3 (CyHV-3) DNA	1	0	0	1	1

in sediments of natural lakes and ponds					
Skin mucus of Cyprinus carpio inhibits cyprinid herpesvirus 3 binding to epidermal cells	1	0	0	0	0
Studying the Genetics of Resistance to CyHV-3 Disease Using Introgression from Feral to Cultured Common Carp Strains	0	0	1	0	0
Survival Rate and Immunological Responses of Mirror Carp Selective Breeding Generations to CyHV-3	1	0	0	0	0
The role of live fish movements in spreading koi herpesvirus throughout England and Wales	1	0	0	0	0
Transmission of Cyprinid herpesvirus-3 (CyHV-3) from goldfish to naïve common carp by cohabitation	1	0	1	1	2
Vaccination against Koi Herpesvirus Disease	1	0	1	0	1
Vaccination of plasmid DNA encoding ORF81 gene of CJ strains of KHV provides protection to immunized carp.	0	0	1	0	0
Validation of a KHV antibody enzyme-linked immunosorbent assay (ELISA)	0	1	0	0	0
Validation of a serum neutralization test for detection of antibodies specific to cyprinid herpesvirus 3 in infected common and koi carp (Cyprinus carpio)	0	0	1	0	0
Susceptibility of koi x crucian carp and koi x goldfish hybrids to koi herpesvirus (KHV) and the development of KHV disease (KHVD)	0	0	1	0	0
Investigation on the diagnostic sensitivity of molecular tools used for detection of koi herpesvirus	0	0	1	1	1
Resistance of common carp (Cyprinus carpio L.) to Cyprinid herpesvirus-3 is influenced by major histocompatibility (MH) class II B gene polymorphism	0	0	1	0	0
Antibody response of two populations of common carp, Cyprinus carpio L., exposed to koi herpesvirus	0	0	1	0	0
Susceptibility of koi carp, common carp, goldfish, and goldfish x common carp hybrids to cyprinid herpesvirus-2 and herpesvirus-3	0	0	1	0	0
Detection of carp interstitial nephritis and gill necrosis virus in fish droppings	0	0	1	0	0

Protection of cultured Cyprinus carpio against a lethal viral disease by an attenuated virus vaccine	0	0	1	0	0
Efficient vaccine against the virus causing a lethal disease in cultured Cyprinus carpio	0	0	1	0	0
Type I interferon responses of common carp strains with different levels of resistance to koi herpesvirus disease during infection with CyHV-3 or SVCV	0	0	0	1	0
Intestinal barrier of carp (Cyprinus carpio L.) during a cyprinid herpesvirus 3-infection: Molecular identification and regulation of the mRNA expression of claudin encoding genes	0	0	0	1	0
Generation and functional evaluation of a DNA vaccine co-expressing Cyprinid herpesvirus-3 envelope protein and carp interleukin-1 beta	0	0	0	1	0
Total	**39**	**16**	**36**	**14**	**43**

Appendix 4 – Phase 2 Meeting

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Biosecurity and Control strategy options against Cyprinid Herpesvirus 3 (CyHV-3) in Australia in relation to the potential release of the virus as a biocontrol agent.

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16 May 2019

Contents

1.0 Biosecurity Risk Analysis of CyHV-3

This process helps identify the areas which require the greatest biosecurity investment to deliver maximum protection to the farm, or hobbyist pond and tanks, from incursion of CyHV-3 and disease impacts of Koi Herpesvirus Disease (KHVD).

Figure 2: Four steps of biosecurity risk analysis

1.1 Identify the hazards

In this project, the hazard being assessed is CyHV-3. Other hazards are outside the scope of the project, but can be considered using this framework by koi owners on another occasion.

CyHV-3 has been identified as a hazard because it has caused adverse impacts on fish health and production internationally. Additionally, this disease has not occurred in Australia (presently considered exotic), and should it be released as part of a carp control program, koi owners stock will be placed at an increased risk.

1.2 Risk Assessment of hazards

To assign a level of risk to a hazard, two factors need to be determined – the likelihood of exposure on your site and the consequence(s) of it occurring on your site. Veterinarians with an interest in aquatic species will be able to assist with this section.

Likelihood can be estimated by considering the transmission pathways necessary for entry of a pathogen (disease causing agent-CyHV-3), and for exposure of your fish. For example, should CyHV-3 be released into the wild, the likelihood of exposure via water, when using water sources that contain wild carp will be 'certain', if the carp control program seeks to use the virus to control carp in all wild waters. If your facility is using chlorinated town water as the source, then the entry of CyHV-3 via this route, would be considered 'remote' as chlorination would be expected to deactivate the virus.

Similarly, pathways involving entry of infected (either clinical (expressing signs of disease) or sub-clinical (not exhibiting any obvious external signs of sickness)) live fish have the highest likelihood of causing exposure because they may shed the pathogen into your naïve, clean koi population.

The likelihood rating for exposure will vary depending on:

- the properties of the pathogen
- the occurrence of the pathogen outside the site or in nearby sites and
- the possible pathways onto the site

Likelihood ratings and descriptors are shown in Table 1.

Table 13: Assessment of disease likelihood

Rating	Descriptor
Remote (1)	Occurs less than once in 20 years
Unlikely (2)	Occurs not more than once in 5-20 years
Possible (3)	Occurs not more than once in 3-5 years
Likely (4)	Occurs not more than once in 2 years
Certain (5)	Occurs every year

Consequence can be estimated by considering the impact(s) of the disease (where the pathogen has damaged tissues of the host fish) on the productivity/health of your fish population and enterprise. The consequences could include multiple aspects (e.g. mortality, reduced growth or food conversion, reduced product quality, reduced market access, lost sales, emotional stress and trauma from loss of a pet, and/or treatment costs).

Consequence ratings and descriptors are shown in Table 2.

Table 14: Assessment of disease consequences

Rating	Descriptor
Insignificant (1)	Impact not detectable or minimal
Minor (2)	Impact is limited to some, not all, units and/or short term only
Moderate (3)	Impact of most populations on site, with increased mortality and/or decreased performance, but not business or hobby ending. Stock loss may result in some emotional stress.
Major (4)	All populations affected. Considerable impact resulting in serious supply constraints, stock loss and financial impact, some emotional trauma.
Catastrophic (5)	All populations affected. Likely complete depopulation of the site and possibly barriers to resumption of production/hobby, highly significant emotional trauma.

Risk estimation—Risk is estimated as a product of likelihood and consequence, resulting in risk ratings of 1–25. Risks are highest when both likelihood and consequence are high. However, the risk may be low even if the consequence is 'catastrophic', as the likelihood may be 'remote' for that particular circumstance; similarly, even if the likelihood is 'certain', the consequence may be 'insignificant'. Risk ratings can be determined by applying estimates of likelihood (where 1 is remote and 5 is certain) and consequence (where 1 is insignificant and 5 is catastrophic) to the risk matrix provided below in Table 3.

Table 15: Risk estimation matrix

Likelihood rating	Consequence rating: Insignificant	Minor	Moderate	Major	Catastrophic
Remote	1	2	3	4	5
Unlikely	2	4	6	8	10
Possible	3	6	9	12	15
Likely	4	8	12	16	20
Certain	5	10	15	20	25

The need for risk mitigation management responses flows from the risk estimation in Table 3 to the responses outlined in Table 4.

Table 16: Risk levels and management responses

Risk level	Explanation and management response
1-2 Negligible	Acceptable level of risk. No immediate action required.
3-5 Low	Acceptable level of risk. On-going monitoring may be required.
6-10 Medium	Unacceptable level of risk. Active management is required to reduce the level of risk.
12-15 High	Unacceptable level of risk. Intervention is required to mitigate the level of risk.
16-25 Extreme	Unacceptable level of risk. Urgent intervention is required to mitigate the level of risk.

Table 17: Application of risk assessment to koi industry risk activities.

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Water source (and entrained organisms in water) to farm/pond/tank	Surface waters that communicate with wild carp populations including areas connected only during flooding. Crustaceans, molluscs and planktons may potentially be carriers of CyHV-3 and transmit pathogens to koi.	Certain (5)	Catastrophic (5)	**Extreme (25)**
Introduction of new koi onto premise	High potential for some koi to be infected (sub-clinical carrier).	Likely (4)	Catastrophic (5)	**Extreme (20)**
Introduction disease from non-target species onto the facility/premise	Non-target species may become carriers of CyHV-3.	Possible (3)	Catastrophic (5)	**High (15)**
Introduction of disease from birds, vermin and pets/farm animals.	Regurgitated/digested CyHV-3 infected carp/koi may be infective. Birds, vermin and pet/farm animals may come into contact with CyHV-3 infected fish and/or contaminated material and bring infected material back to koi ponds/tanks.	Possible (3)	Catastrophic (5)	**High (15)**
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	People and equipment that have come into contact with water contaminated or fish infected with CyHV-3 may potentially be vectors for the disease	Possible (3)	Catastrophic (5)	**High (15)**
Introduction of disease (CyHV-3) through feed.	No feeding of raw fish. Only extruded pellets are fed. A slight potential that extruded feed could come into contact with a source of the virus.	Unlikely (2)	Catastrophic (5)	**Medium (10)**

B: Risks for pathogen (CyHV-3) spread within a koi facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Transmission of virus through aerosol/ water movement within a koi facility	Movement of the pathogen may occur via aerosol movement. Water which has not been treated may have come into contact with CyHV-3 infected fish which may allow for the virus to transmit to multiple tanks/ponds.	Likely (4)	Catastrophic (5)	**Extreme (20)**
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	People and equipment that have come into contact with water contaminated or fish infected with CyHV-3 may potentially be vectors for the disease. Equipment not dedicated to one fish group.	Possible (3)	Catastrophic (5)	**High (15)**
Movement of disease by birds, vermin, pets/farm animals between ponds/tanks	Diseased/latently infected fish are present on site.	Likely (4)	Catastrophic (5)	**Extreme (20)**
Spread of disease (CyHV-3) through feed.	Feed may potentially become contaminated with CyHV-3 and act as a vector for disease. Elevated opportunity for contamination of feed once virus is established on-site.	Possible (3)	Catastrophic (5)	**High (15)**

C: Risk to other koi facilities of release of CyHV-3 from an infected facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Discharge of pond water into storm water drainage	CyHV-3 may be present and viable in water from a pond containing infected koi (whether clinically, of sub-clinically infected).	Likely (4)	Catastrophic (5)	**Extreme (20)**
Discharge of pond water into wastewater (sewer) source	CyHV-3 may be present and viable in water from a pond containing infected koi (whether clinically, of sub-clinically infected).	Likely (4)	Insignificant (1)	**Low (4)**
Release of CyHV-3 through carriage of infected fish, or water which contains CyHV-3, by birds, vermin, pets/farm animals contacting infected site and	Birds, vermin, pets/farm animals have contact/access with the infected pond water and fish population.	Possible (3)	Catastrophic (5)	**High (15)**

moving to water bodies outside of the koi site.				
Disposal of mortalities	Fish mortalities are not promptly removed, buried, burnt, or bagged.	Possible (3)	Catastrophic (5)	**High (15)**
Release of the disease by people and equipment to wild riverine water source	People and equipment that have come into contact with water contaminated by, or fish infected with, CyHV-3 may potentially be vectors for the disease	Possible (3)	Catastrophic (5)	**High (15)**

D: Risks of pathogen (CyHV-3) spread at auctions and koi shows

Risk activity	**Assumption**	**Likelihood**	**Consequence**	**Risk rating**
Exposure through mixing of koi populations	Sub-clinically infected koi at shows/auctions will come into contact with uninfected koi. Purchasing of infected koi increases risk of spread of disease among existing stock at show, and upon return of stock to koi owner's site.	Certain (5)	Catastrophic (5)	**Extreme (25)**
Exposure to CyHV-3 through contaminated equipment and/or people	People and equipment will come into contact with water contaminated by, or fish infected with, CyHV-3.	Likely (4)	Catastrophic (5)	**Extreme (20)**

1.3 Risk management measures

Control measures for risk activities identified in Table 5 are detailed below. Advantages and disadvantages of each management measures are listed alongside the management measures. Management options in bold text represent the best practice option which FFVS recommend, options in plain text are alternatives that have been identified in the literature review and from general biosecurity knowledge.

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

Risk activity	Option number	Risk Management	Options	Advantages	Disadvantages
Water source (and organisms entrained within) to farm/pond/tank	1	Selection of water source(s)	**a) Use of town water (for small volume applications)**	Easily accessible. High efficacy and reliability.	Not suitable for large volume requirements due to cost and availability. Water required to be dechlorinated prior to use.
			b) Use of lake/river/small dam water	Ability to access significantly larger volumes of water cf. town water.	High risk of contamination with pathogens (i.e. CyHV-3) and potential disease carriers (i.e. carriers of CyHV-3). Would require additional decontamination steps for secure use.
			c) Use of bore-water (for large volume applications)	Ability to access significantly larger volumes of water cf. town water. Free from fish pathogens and carrier organisms.	Water quality may vary significantly or be incompatible for fish and require pre-treatment prior to use to allow degassing, pH adjustment and address mineral composition.
	2	Disinfection/ deactivation of	**a) Disinfect all incoming water with UV light at 4.0×10^3 µWs/cm2**	Effective at reducing plaque count by 100%. Automated once installed.	Cost of equipment dependent on flow requirements; requires pre-filtration of water to under 15 microns.

	CyHV-3 in incoming water	b) Disinfect all incoming water with chemical disinfectants at concentrations listed in Table 6.	Effective in reducing CyHV-3 infectivity.	Cost; requirement to stock potentially hazardous chemicals; handling potentially hazardous chemicals, risks to fish if residual chemicals flow into fish tanks. Increased management requirement to dose water and decontaminate prior to use
		c) Heat all incoming water to 50ºC for 1 minute	Effective in reducing CyHV-3 infectivity	High energy cost; equipment cost; management required to heat and cool water to avoid given fish a thermal stress from rapid change in temperature.
		d) Ozone at 0.5mg/L total residual oxidants (TRO) concentrations of ozone for 15 seconds	May provide alternative to UV to disinfect water against CyHV-3.	Research was not performed on CyHV-3; cost of equipment and monitoring; potentially toxic to fish, humans, and other animals.
		e) Hold all incoming surface water (eg river/dam) in fish free tank for 3 days **f) Hold all incoming surface water for 7 days.** This includes a safety factor in case virus deactivation is delayed.	Cheap to perform. Minimum holding of 3 days has been described in the literature to be effective for surface waters.	Requires at least 2 tanks to allow batch supply into the system. Requires manual operation, creating room for human error and introduction of water which may not be fully decontaminated.
3	Deactivation of CyHV-3 with bacteria isolates	a) Add bacteria isolates with anti-CyHV-3 properties to the incoming water and hold for 3-7 days	May decrease infectivity of CyHV-3 if it is present in water	Unlikely to be efficacious. Cost is unlikely sustainable. Bacteria may lose expression of the anti-CyHV-3 properties after repeated cultures.

4	Management of water quality	**a) Testing and record: Dissolved oxygen (DO), Temperature, pH, Salinity, Total Ammonia Nitrogen (TAN), algal blooms to maintain good water quality. Maintain clean ponds.**	Provides early warning of aberrant water quality to allow operator opportunity to correct situation prior to stressing fish.	Cost of meters. Requirement for operator time commitment.
5	Prevention of potentially infected planktons, molluscs and crustaceans entry into ponds/tanks.	**a(i) Filtration with 5 micron filters**	Reduces risk of introducing potentially infected organisms into the system	Equipment required for filtration.
		a(ii) Using bore-water or town-water as the water source		Requires access to bore or town water.
		b) Avoid accidental stocking of potentially infected planktons, molluscs and crustaceans using chemicals/disinfectants such as sodium hypochlorite to kill and disinfect organisms in source water.		Requires chemical usage and handling
6	Prevention of wild carp entry into ponds/tanks.	**a) Ensure that adequate filtration is in place to ensure that no carp/eggs can enter the system through intake water.**	Reduces risk of accidental stocking of wild carp	Cost of filtration
		b) Ensure adequate fencing/netting are in place to prevent bird access to ponds/tanks.		Cost of netting, loss of aesthetic value-impact on appearance of pond

Introduction of new koi onto premise	7	Sourcing new fish	**a(i) Only source fish from specific pathogen free (SPF) (free of CyHV-3) certified sites/facilities where possible (none presently certified- require establishment if CyHV-3 released).**	SPF facilities offer high level assurance of freedom from CyHV-3 attained through surveillance, testing and implementation of rigorous biosecurity barriers to maintain disease free status.	None currently available. Cost and time required to maintain and obtain SPF status.
			a(ii) Only source fish from disease free facilities with known histories.	Available presently. Low cost.	Low confidence of declared status as not underpinned by surveillance data and biosecurity measures. Higher risk of accidental introduction of carrier fish.
			b) Only source fish that have been vaccinated for CyHV-3.	Reduces risk of koi being infected with CyHV-3	Vaccines are currently not available in Australia. Varying efficacies have been reported with vaccines up to ~80-90% protection. No trial vaccines thus far are 100% protective.
			c) Only stock fish that have recent CyHV-3 free status certifications from appropriate diagnostic tests.	Reduces the risk of stocking CyHV-3 infected koi.	Likely cost associated with testing of koi to obtain certifications. Potential for false negative results. Need for terminal sampling to get most sensitive test result, excludes most sensitive approach from individual fish purchase scenario.
	8	Duration of quarantine period	**a(i) Quarantine all new and returning fish upon arrival/return for a minimum of three (3) weeks, provides greater safety margin**	May provide sufficient time for development of clinical disease to be observed, allowing owner to not mix the affected fish, with the rest of the stock on site.	Cost of additional facilities to maintain stock and time involved in quarantining.

	a(ii) Quarantine all new and returning fish upon arrival/return for a minimum of two (2) weeks		
Cohabitation with sentinel koi	**b(i) Quarantine all new and returning fish with thirty (30) sentinel koi for the duration of the quarantine period**	Allows for larger sample populations and for lethal (most sensitive technique) sampling of koi.	Cost involved in maintaining and purchasing of sentinel koi. Cost in sampling and testing cost.
	b(ii) Quarantine all new and returning fish with ten (10) sentinel koi for the duration of the quarantine period	Lower cost to perform than maintaining 30 sentinel fish. Allows for lethal sampling of koi.	Cost involved in maintaining and purchasing of sentinel koi. Cost in sampling and testing cost. Increase chance of false negative results compared to sampling of 30 sentinel fish.
Quarantine temperature ranges	**c(i) Quarantine all new and returning fish upon arrival/return at permissive temperature for KHVD (16 °C – 28 °C).**	Allows for clinical disease to develop and manifest.	May require active cooling/heating.
	c(ii) Quarantine all new and returning fish upon arrival/return at permissive temperature for CyHV-3 transmission (12°C – 28 °C).	Allows for transmission of disease	Subclinically affected koi may not develop clinical disease or be detected by testings.
Monitoring of quarantine koi	**d(i) Observe quarantine fish daily and record and document any abnormal observations or test findings in a journal or an appropriate recording system.**	Allows for monitoring and tracking of changes and abnormalities	Time involved in monitoring.

	d(ii) No observation of quarantine fish.	Allows for monitoring of abnormalities	No ability for early detection of disease. Increases risk for other fish.
Selection of quarantine location	**e(i) Monitor all new and returning fish in a dedicated quarantine system for signs of disease.**	Reduces risk of disease transmission from new/returning fish to existing stock.	Requires access to dedicated quarantine system.
	e(ii) Monitor all new and returning fish in the same system as existing stock for signs of disease.	No additional facility/system is required.	No ability to control disease introduction. No biosecurity barriers in place. If infected fish enters, then transmission to all other stock is likely.
Additional quarantine barriers	**f) All quarantine equipment remains in quarantine area.**	Reduces risk of disease transmission from new/returning fish to existing stock through potential viral carriage by equipment.	Cost of additional equipment.
	g) Where no alternative exists, and the use of non-dedicated equipment is required, non-dedicated equipment should be disinfected with chemicals described in Table 6 prior to use.		Slight increased risk of disease transmission/ spread to existing stock compared to using dedicated equipment only, where decontamination protocol may be ineffective. Cost of sanitiser.

	Deliberate stressors during quarantine	**h(i) Apply transport, temperature, handling and sampling stress to new and/or sentinel koi during the quarantine period.**	May increase rate of CyHV-3 detection in testing, and expression of disease through reactivation of latent/ subclinical disease.	May increase incidence of secondary diseases that may otherwise have not developed. Cost of energy and equipment to modify temperature environment.
		h(ii) No deliberate stressors are used to challenge fish.	May reduce the incidence of secondary diseases. Reduced cost, as no energy requirement to heat/chill water.	Latent/ subclinical disease may not be detected. The chances of false negative testing results are increased.
9	Disease investigation/surveillance of sick/ freshly dead quarantined fish	**a(i) Sample all new and returning fish non-lethally (mucus, blood and/or gill swab samples), and all sentinel koi lethally, at the end of the quarantine period by (kidney , spleen, gills) and perform at least three (3) molecular diagnostic tests (i.e. nested PCR, real time PCR, qPCR) for CyHV-3 and histology.**	Increased ability to detect subclinically affected/ latent carriers of diseases	Cost involved in testing all the fish.
		a(ii) If any clinical signs of sickness presents, sample all and returning new fish (and all sentinel koi) at the end of the quarantine period non-lethally (mucus, blood and/or gill swab samples) and perform at least three (3) molecular diagnostic tests (i.e. nested PCR, real time	Targeted screening of typically affected disease fish. Reduces cost of diagnostics and increases detection rates of diseased animals.	Less likely to detect subclinical/latent infections. Will likely still yield good sensitivity for detection.

		PCR, qPCR) for CyHV-3 and histology.		
		a(iii) Sample all sick new and returning fish (and all sick sentinel koi) at the end of the quarantine period non-lethally (mucus, blood and/or gill swab samples) and perform at least three (3) molecular diagnostic tests (i.e. nested PCR, real time PCR, qPCR) for CyHV-3.	Targeted selection of diseased fish which are more likely to be positive for diseases. Reduces cost of monitoring.	More likely to miss subclinical/latent infections.
		b) Perform pond side tests with an ELISA lateral flow device test kit to detect suspected CyHV-3 infected fish during quarantine	Ability to access results fast (approximately 15 minutes).	Lower diagnostic sensitivity cf. PCR. Test not currently available in Australia. Appears to be only useful in acute stages of the disease or in recently deceased fish. May yield an increased number of false negative results.
10	Health status of new fish	**a(i) Ensure health status is equal to, or higher, than the existing stock population through: targeted testing for relevant pathogens (eg CyHV-3); selection from populations that have not exhibited disease outbreaks; appropriate import/translocation permits**	Significantly reduces risk of translocating CyHV-3 by only moving stock from a tested free population. If performed without use of quarantine at destination site, it is potentially cheaper	Cost of monitoring and certification. If performed without quarantine, is likely to have higher risk of sub-clinical fish evasion from detection.

			in place for stock movement. New fish are only to be introduced into the facility once all diagnostic test results have been received and are negative for CyHV-3.		
			a(ii) Ensure that the health status of all new fish is adequate before stocking with existing stock.	Cheaper without using diagnostic tests	As sub-clinical carriage is common, visual observation alone will not detect them.
Introduction disease from non-target species onto the facility/premise	11	Reduction of risk associated with cohabitation/stocking of potential non-target species	a(i) Do not cohabitate koi or carp with goldfish, silver carp, fathead minnow, grass carp, blue black ide, Ancistrus sp., koi/carp hybrids, Topmouth gudgeon (Pseudorasbora parva) which been described to potentially harbour the virus.	Reduces risk of CyHV-3 transmission to koi from potential carriers of the virus.	Potential risk of transmission is still present on the site requiring other biosecurity barriers to be robust, such as aerosol control, and equipment decontamination.
			a(ii) Avoid stocking goldfish, silver carp, fathead minnow, grass carp, blue black ide, Ancistrus sp., koi/carp hybrids, Topmouth gudgeon (Pseudorasbora parva) which been described to potentially harbour the virus on site.	Avoids risk of CyHV-3 transmission to koi from potential carriers of the virus	Inability to stock potential carriers on site, which may be desirable aesthetic in mixed ornamental fish collections.

Introduction of disease from birds, vermin and pets/farm animals.	12	Reduction of access of vermin (i.e. birds, rodent) and pets/farm animals to koi ponds/ tanks/ associated infrastructure.	**a) Erect bird fences to eliminate birds/pets from entering ponds. Control rodents by good feed storage and bait stations.**	Reduces risk of potential spread of disease between systems, and entry of diseases from external area.	Cost of equipment and maintenance. Loss of aesthetic value of pond.
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	13	Minimisation of contact with potentially contaminated equipment	**a) Disinfect all equipment with chemicals at doses stated in Table 6 after each use.**	Reduces risk of potential spread of disease between systems, and entry of diseases from external area.	Cost of chemicals. Requirement to handle potentially hazardous chemicals.
			b(i) Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should not be brought into the facility. Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry	Reduces risk of potential spread of disease between systems	Cost of chemicals. Requirement to handle potentially hazardous chemicals. Increased risk of disease transmission compared to using dedicated equipment only.
			b(ii) Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should be thoroughly cleaned and disinfected with chemicals		

		detailed in Table 6 prior to entry to the facility		
14	Minimisation of risks associated with people/visitors/staff that may have been exposed to potentially contaminated water or diseased fish.	**a) Disinfect hands and footwear upon entry (and exit) to a koi facility, and between separate areas of facility**	Reduces risk of potential entry and spread of CyHV-3 between systems	Cost of chemicals. Requirement to handle potentially hazardous chemicals.
		b(i) Staff/visitors/owners must wear freshly laundered clothes each day prior to entry into a koi facility.	Reduces risk of potential entry and spread of disease between systems	Inconvenience to visitors
		b(ii) Staff/visitors/owners must change into freshly laundered clothes and site provided footwear prior to entry into a koi facility.	Reduces risk of potential spread of disease between systems	
		c(i) All visitors/contractors/researchers must complete the visitor log, biosecurity questionnaire, induction and sign declaration prior to being considered for access. Visitors who are assessed as high risk will not be allowed entry	Reduces risk of potential spread of disease between systems	Inconvenience, and may be contrary to the desired aim of hobbyist to show others their systems and stock.
		c(ii) All visitors/contractors/researchers must be aware of the biosecurity required prior to being granted entry.		

			d) Visitors must be accompanied by a staff member/owner at all times	Reduces risk of potential spread of disease between systems	
			e) Visitor vehicles to be parked in dedicated parking area, preferably remote to fish stock and fish movement loading area	Reduces risk of potential spread of disease between systems	
			f) Visitors are discouraged from touching walls tank/pond structures, water and/or fish.	Reduces risk of potential spread of disease between systems	May be inconvenient where buyers seeking to examine stock
Introduction of disease (CyHV-3) through feed.	15	Avoidance of feeding potentially contaminated food.	**a) Feed sanitised food (i.e extruded pellets) only where possible. Do not feed materials that has been potential contaminated with CyHV-3.**	Reduces risk of introducing/transmitting disease from infected food to koi through the oral route.	Status of sites of feed storage may not be easily known.
			b) Do not feed raw fish to koi.	Reduces risk of introducing/transmitting disease from infected food to koi through the oral route.	

B: Risks for pathogen (CyHV-3) spread within a koi facility

Risk activity	Option number	Risk Management	Options	Advantages	Disadvantages
Dissemination of pathogen (CyHV-3) from fish movement between tanks/ponds	1	Management of fish movement within a facility.	a) Record all movement of all fish on site, between areas	Allows for tracking of fish movement in the event of an outbreak	

Transmission of virus through aerosol/ water movement within a koi facility	2	Management of water between systems.	**a) Where water is recirculated, ensure that appropriate measures (i.e with UV and/or ozone) are made to disinfect water to ensure that only sanitary water is recirculated**	Reduces risk of spreading diseases across populations	Equipment and maintenance costs.
	3	Reduction of aerosol spread between ponds/tanks.	a) Ensure that all doors between facilities/sites remain closed at all times where possible.	Reduces risk of spreading CyHV-3 across populations via aerosol	May not be applicable/ possible for outdoor systems.
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	4	Minimisation of contact with potentially contaminated equipment	**b) Access to quarantine zones is avoided where possible**	Reduces risk of potential spread of disease between systems	
Infection among existing stock	5	Detection of latently infected fish	a(i) Routinely sample skin mucus and gill mucus every six months/year and perform 2 PCR methods For ELISA tests, at least ten blood samples from separate individual koi are required. **a(ii) Sample all sick koi/fresh mortalities/euthanised sick koi for histopathology (where appropriate) and for a minimum of two PCRs (i.e. nested PCR, real time PCR, qPCR)**	Enables detection of disease to enable mitigation strategies.	Cost of active surveillance (screening apparently healthy fish), and passive surveillance (screening sick/dead fish)

		b) Apply stressors including transport, handling, temperature fluctuations and sampling stress 3-6 days prior to collection of samples.	Increases detection rate of CyHV-3.	May induce secondary opportunistic diseases in stock
6	Vaccination of existing stock	a(i) Vaccinate all existing and new fish with a live attenuated vaccine	Provides approximately 80% survival rates at best. Protection may persist for 13 months (64% survival), requires re-vaccination	All vaccinated fish will test positive for CyHV-3. Live attenuated strain may mutate and become pathogenic and cause KHVD. Vaccinated fish may transmit live attenuated strains of the virus to other naïve fish (i.e. to wild carp populations). Vaccine is currently not commercially available, nor registered with APVMA in Australia.
		a(ii) Vaccinate all fish with an ORF-25 or other similar DNA intramuscular vaccine	Provides approximately 40% survival rates at best. No horizontal transmission (accidental vaccination of other fish). Vaccinated fish will not test positive for CyHV-3 by antigen testing. Vaccine will not mutate and become virulent.	Duration of protection is unknown. Requires three injections to be effective. Handling involved. Currently not available, nor registered with APVMA in Australia.
		a(iii) Vaccinate all fish with an ORF-81 DNA or other similar oral vaccine	Provides approximately 50% survival rates at best. No horizontal transmission (accidental vaccination of other fish). Vaccinated fish will not test positive for CyHV-3 by antigen testing. Does not require handling of the fish. Vaccine will not mutate and become virulent.	Duration of protection is unknown. Will favour 'greedier' fish that consume more feed. Currently not available, nor registered with APVMA in Australia.

a(iv) Vaccinate all fish with a liposome vaccine	Provides approximately 75% survival rates at best. No horizontal transmission (accidental vaccination of other fish). Vaccinated fish will not test positive for CyHV-3 by antigen testing. Does not require handling of the fish. Vaccine will not mutate and become virulent.	Duration of protection is unknown. Will favour 'greedier' fish that consume more feed. Currently not available, nor registered with APVMA in Australia.
a(v) Administration of oral anti-CyHV-3 IgY incorporated diets	Provides approximately 15-40% survival rates at best. No horizontal transmission (accidental vaccination of other fish). Fish given the anti-CyHV-3 diet will not test positive for CyHV-3 by antigen testing. Does not require handling of the fish. IgY diet will not mutate and become virulent.	Not a vaccine. Duration of protection is unknown. Will favour 'greedier' fish that consume more feed. Currently not available, nor registered with APVMA in Australia.
b) Avoid stressing (transport, temperature fluctuation, handling) fish where possible and avoid the use of chemicals such as organophosphate insecticides and/or water potentially contaminated with organophosphates. Seek advice from a veterinarian with expertise in koi disease prior to attempting to treat parasitic infections.	Reduces risk of immunosuppression due to stress factors.	Cost of testing source water for contaminants.

Dissemination of disease (KHV) after a disease outbreak to other ponds/tanks.	7	Prevention of spread of disease between systems/site and to another systems/site	**a(i) In the event of a material disease, a pond/system all fish are culled, and a decontamination process (i.e. hydrated lime (CaOH) application and drying of ponds in sunlight for a minimum of 7 days) must be undertaken prior to restocking. Decontamination must be undertaken in consultation with, or under instruction from, the relevant state government biosecurity agency to ensure compliance with biosecurity obligations or with a competent veterinarian. Fish movement is restricted for a minimum of one year (or until water temperatures fall below permissible ranges the following year). No new fish are permitted to be introduced into the site/fishery during the restriction period.**	Significantly reduces risk of disease outbreaks in the future due to persistence of latent/low level infections.	Requires culling of stock
			a(ii) In the event of a material disease, additional biosecurity measures (signage, footbath stations, hand disinfection stations, equipment disinfection points) are placed around the site/fishery. Fish movement is restricted for a minimum of one year (or until water temperatures fall below permissible ranges the	No culling of collection/stock required.	Latently affected, apparently healthy, carriers of CyHV-3 likely to establish in the population.

			following year). No new fish are permitted to be introduced into the site/fishery during the restriction period.		

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility

Risk activity	Option number	Risk Management	Options	Advantages	Disadvantages
Discharge of pathogen (CyHV-3) into the environment	1	Management of effluent discharge	a(i) Hold all untreated/filtered effluent water for 3 days prior to discharge.	Relatively low cost to perform if sufficient space and ability to hold water is present. Minimum holding of 3 days has been described in the literature.	Only applicable for untreated and unfiltered water. Difficult to hold large volumes of water. Requires manual operation, creating room for human error and discharge of water which may not be fully decontaminated. Requires anti-CyHV-3 bacteria to be present to be effective. May be difficult to hold large quantities of water for extended periods.
			a(ii) Hold all untreated/filtered effluent water for 7 days prior to discharge. This includes a safety factor in case virus deactivation is delayed.		
			a(iii) Disinfect all effluent water with chemicals at dosages described in Table 6 prior to discharge. Decontamination, testing, or holding of water prior to discharge may be required.	Deactivates virus and reduces risk of spread outside of facility.	Requirement to handle potentially hazardous chemicals.
			b) Ensure that all effluent discharge and their associated	Reduces risk of contamination of the source water supply	

			aerosol are separate from intake water and supply.		
Release of disease from mortalities	2	Appropriate disposal of mortalities	a) Discard all mortalities and sick animals appropriately into sealed plastic bag to land-fill or deep onsite burial or by incinceration. Never release koi into waterways.	Reduces risk of wildlife access to dead and potentially infected fish. Reduces risk of seepage of potentially infected material into water sources.	Handling of fire safety issues and odour emission issues.
			b) Fish mortalities are recorded and removed daily and disposed of by a method approved by the relevant authority, which ensures no risk of release of pathogens from the dead stock into waterways, or access for scavenger birds or animals (e.g. pigs, foxes, water rats) that could spread a disease.	Reduces risk of wildlife access to dead and potentially infected fish and subsequent movement of infected material into ponds/ waterways/ tanks. Ability to record and track mortalities.	
Release of the disease by people and equipment	3	Management of people	**a) Disinfect hands and footwear upon exiting the farm/site.**	Reduces risk of potential spread of disease from within the site to areas outside the site	Handling of potentially hazardous chemicals.
	4	Management of equipment	a(i) Dedicated equipment should be labelled and maintained for use exclusively on site. Dedicated equipment should not be removed from site and use for other purposes.	Reduces risk of spreading diseases out of facility through contaminated equipment	Cost of maintaining dedicated equipment

			a(ii) Dedicated equipment should be labelled and maintained for use exclusively on site. Use of dedicated equipment off site is avoided whenever possible. If no alternative exists, then a thorough cleaning and disinfection protocol must be followed, prior to entry		Cost of maintaining dedicated equipment. Handling of potentially hazardous chemicals.

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows

Risk activity	Option number	Risk Management	Options	Advantages	Disadvantages
Infection from exposure to contaminated water or disease fish at the auction/show	1	Additional disinfection protocols at koi shows/auctions	**a) For koi shows/auctions, additional disinfection with hydrogen peroxide based solution (i.e. Huwa-San©) at 60mg/L can be added into tanks, with care required in higher water temperatures (>20°C) if holding for longer than one hour**	Shown to be able to prevent CyHV-3 transmission between infected fish and naïve fish sharing the same water	Requirement to handle chemicals; increases water temperature when used through exothermic reaction with water; may potentially be hazardous to the gills of fish at high temperatures. Huwa-San © is not registered for use in Australia for aquatic animals.
	2	Avoidance of certain show formats	**a) Where possible, do not cohabitate koi or share water with koi from different systems/facilities/premises/ponds/tanks together with other koi.** **Where possible, adopt the English style layout for all koi shows to prevent risk of horizontal transmission of**	Reduces risk of dissemination of disease through contact with many naive populations from different sources.	Japanese style koi shows would not be permitted.

		CyHV-3 through contact with infected fish.		
		b) Where koi from different systems or facilities must be cohabitated or water must be shared, a hydrogen peroxide based solution (i.e. Huwa-San©) at 60mg/L must be added into tanks to prevent spread of CyHV-3. Caution must be exercised in high temperature waters especially if holding for longer than one hour.	Shown to be able to prevent CyHV-3 transmission between infected fish and naïve fish sharing the same water	Hydrogen peroxide can be extremely hazardous to fish at high water temperatures. It releases oxygen, water and heat when in water and can cause gill damage. Huwa-San © is not registered for use in Australia for aquatic animals.
3	Disinfection and disposal of transport water returning from auctions/shows	**a) Disinfect all waters used for transport during the auction/show with permitted disinfectants described in Table 6 prior to discharge.**	Reduce risk of introducing potentially contaminated waters to stock waters.	
		b) Do not discharge water used for the transport of fish from the auction/show into ponds/tanks/water supply.	Prevents introduction of disease from potentially contaminated waters.	

			c(i) Hold all water used for transport during the auction/show that has not been disinfected or filtered for 3 days prior to discharge	Reduce risk of introducing potentially contaminated waters to stock waters.	
			c(ii) Hold all water used for transport during the auction/show that has not been disinfected or filtered for 7 days prior to discharge		
Showing/auctioning of infected koi	4	Screening of koi prior to shows/auctions	**a(i) Current veterinary health certificates and diagnostic results must be obtained prior to placing koi into shows/auctions**	Minimises risk of spreading diseases at shows/auctions.	Cost of diagnostics and veterinary interpretation.
			a(ii) Koi fish that visibly appear sick are not permitted to attend shows/auctions		Increased risk of potentially showing/auctioning of subclinical carriers of CyHV-3.
	5	Limiting of koi permitted to shows/auctions	b) Only vaccinated koi are permitted to attend shows/auctions.	Minimises risk of spreading diseases at shows/auctions.	No vaccines currently available nor registered with APVMA.
			c) Koi from facilities that have a recent history of CyHV-3 are not permitted to attend shows/auctions for a minimum of one year (until temperatures fall below permissible temperatures the following year).	Allows early participation back in shows after an infection event. Slightly reduces risk of spreading diseases at shows/auctions.	Carrier fish may still be moved between facilities and risk spreading the disease.

		d) Koi from facilities with a history of CyHV-3 exposure are not permitted to attend shows/auctions unless all stock are culled, and facilities are disinfected and restocked with certified CyHV-3 free stock.	Minimises risk of spreading diseases at shows/auctions.	Will require complete destocking of collection and disinfection of whole facility. High cost, loss of genetic stocks.
6	Reduction of transmission risks associated with equipment usage	**a) Sharing of equipment such as nets is prohibited. Where no alternative exists, thoroughly disinfect equipment prior to use.**	Significantly reduces risk of transmission of virus from contaminated equipment	Cost of separate equipment.
		b) Disinfect all equipment between use.	Reduces risk of transmission of virus from contaminated equipment	Requires the use of chemicals at shows.
7	Reduction of cross contamination by people	**a) Contact with water, equipment or fish during shows/auctions is discouraged (signs, physical barriers).**	Reduces risk of transmission of disease through fomites.	Difficult to regulate and maintain.

Appendix 1

Disinfectant	Treatment time	Temperature (15°C)	Temperature (25°C)	Temperature (unspecified)
Iodophor (mg/L)	30 s	200	200	N/A
	20 min	200	200	N/A
Sodium hypochlorite solution (mg/L)	30 s	>400	>400	N/A
	20 min	200	250	N/A
Benzalkonium chloride solution (mg/L)	30 s	60	30	N/A
	20 min	60	30	N/A
Ethyl alcohol (%)	30 s	40	30	N/A
	20 min	30	25	N/A
Free Chlorine (mg/L)	30 min	N/A	N/A	3

Table 18 Chemicals used for disinfection of CyHV-3 adapted from Kasai et al. 2005.

Appendix 5 – Phase 3 Farm options

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Feasibility of Adoption of Biosecurity and Control strategies against Cyprinid Herpesvirus 3 (CyHV-3) for koi industry farmers in Australia in relation to the potential release of the virus as a biocontrol agent.

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Contents

1. Introduction

Cyprinid Herpesvirus 3 (CyHV-3) also known as Koi Herpesvirus (KHV) is a notifiable disease in koi (*Cyprinus carpio koi*) and European carp (*Cyprinus carpio*). CyHV-3 is the pathogen responsible for causing Koi Herpesvirus Disease (KHVD) which is the damaged tissues which result from viral replication. In subclinical infections with CyHV-3, no tissue damage may be evident, hence the fish is not technically diseased. It is however an infected carrier of the virus which could potentially spread to other susceptible fish. In recent years, CyHV-3 has been explored as a possible biocontrol measure for European carp in Australia. This virus has been detected in 33 countries including Canada, Indonesia, Japan, Taiwan, Thailand, Poland, Singapore, United Kingdom, and the United States of America.

The potential release of CyHV-3 into Australian waters would introduce new risks to Australia's koi industry. A literature review and biosecurity options list were compiled to assess and explore potential risk mitigation strategies against CyHV-3. A stakeholder meeting in Sydney was held to explore each biosecurity options identified from the literature review.

It was identified during the meeting by stakeholders that the risks and mitigation strategies against CyHV-3 differs vastly for koi hobbyists and koi farmers. Current Australian koi farmers were contacted regarding the project and a teleconference was held. Only one koi farmer was available for the teleconference. The views in this report may not reflect the adaptability of biosecurity options for the entire industry in Australia.

This report draws from the previous biosecurity options report circulated prior to the meeting. Agreed approaches by industry stakeholder(s) are placed in the column titled "Agreed approach". Where "None" is written, no agreed option from biosecurity options paper was reached by the stakeholder(s). The feasibility, adoptability and likelihood reduction rating of each option are explored in the "Stakeholder feasibility and adoption limitations" column.

The likelihood reduction rating assesses the level of reduction in likelihood of the risk activity after applying the biosecurity option listed in the "Agreed approach" column. Where no agreed approach was reached, the likelihood reduction rating is listed in the suggestion column. Suggestions made by FFVS have taken the feasibility and adoptability of each biosecurity options into account. The overall risk rating after applying the agreed approach or the stakeholder(s) suggested approach to the risk is listed in the "Risk activity" column.

2. Biosecurity Risk Analysis of CyHV-3

This process helps identify the areas which require the greatest biosecurity investment to deliver maximum protection to the farm, or hobbyist pond and tanks, from incursion of CyHV-3 and disease impacts of Koi Herpesvirus Disease (KHVD).

Figure 3: Four steps of biosecurity risk analysis

2.1 Identify the hazards

In this project, the hazard being assessed is CyHV-3. Other hazards are outside the scope of the project, but can be considered using this framework by koi owners on another occasion.

CyHV-3 has been identified as a hazard because it has caused adverse impacts on fish health and production internationally. Additionally, this disease has not occurred in Australia (presently considered exotic), and should it be released as part of a carp control program, koi owners' / farmers' stock will be placed at an increased risk.

2.2 Risk Assessment of hazards

To assign a level of risk to a hazard, two factors need to be determined – the likelihood of exposure on your site and the consequence(s) of it occurring on your site. Veterinarians with an interest in aquatic species will be able to assist with this section.

Likelihood can be estimated by considering the transmission pathways necessary for entry of a pathogen (disease causing agent-CyHV-3), and for exposure of your fish. For example, should CyHV-3 be released into the wild, the likelihood of exposure via water, when using water sources that contain wild carp will be 'certain', if the carp control program seeks to use the virus to control carp in all wild waters. If your facility is using chlorinated town water as the source, then the entry of CyHV-3 via this route, would be considered 'remote' as chlorination would be expected to deactivate the virus.

Similarly, pathways involving entry of infected (either clinical (expressing signs of disease) or sub-clinical (not exhibiting any obvious external signs of sickness)) live fish have the highest likelihood of causing exposure because they may shed the pathogen into your naïve, clean koi population.

The likelihood rating for exposure will vary depending on:

- the properties of the pathogen
- the occurrence of the pathogen outside the site or in nearby sites and
- the possible pathways onto the site

Likelihood ratings and descriptors are shown in Table 1.

Rating	Descriptor
Remote (1)	Occurs less than once in 20 years
Unlikely (2)	Occurs not more than once in 5-20 years
Possible (3)	Occurs not more than once in 3-5 years
Likely (4)	Occurs not more than once in 2 years
Certain (5)	Occurs every year

Table 19**:** Assessment of disease likelihood

Consequence can be estimated by considering the impact(s) of the disease (where the pathogen has damaged tissues of the host fish) on the productivity/health of your fish population and enterprise. The consequences could include multiple aspects (e.g. mortality, reduced growth or food conversion, reduced product quality, reduced market access, lost sales, emotional stress and trauma from loss of a pet, and/or treatment costs).

Consequence ratings and descriptors are shown in Table 2.

Rating	Descriptor
Insignificant (1)	Impact not detectable or minimal
Minor (2)	Impact is limited to some, not all, units and/or short term only
Moderate (3)	Impact of most populations on site, with increased mortality and/or decreased performance, but not business or hobby ending. Stock loss may result in some emotional stress.
Major (4)	All populations affected. Considerable impact resulting in serious supply constraints, stock loss and financial impact, some emotional trauma.
Catastrophic (5)	All populations affected. Likely complete depopulation of the site and possibly barriers to resumption of production/hobby, highly significant emotional trauma.

Table 20: Assessment of disease consequences

Risk estimation—Risk is estimated as a product of likelihood and consequence, resulting in risk ratings of 1–25. Risks are highest when both likelihood and consequence are high. However, the risk may be low even if the consequence is ‘catastrophic’, as the likelihood may be ‘remote’ for that particular circumstance; similarly, even if the likelihood is ‘certain’, the consequence may be ‘insignificant’. Risk ratings can be determined by applying estimates of likelihood (where 1 is remote and 5 is certain) and consequence (where 1 is insignificant and 5 is catastrophic) to the risk matrix provided below in Table 3.

Likelihood rating	Consequence rating: Insignificant	Minor	Moderate	Major	Catastrophic
Remote	1	2	3	4	5
Unlikely	2	4	6	8	10
Possible	3	6	9	12	15
Likely	4	8	12	16	20
Certain	5	10	15	20	25

Table 21: Risk estimation matrix

The need for risk mitigation management responses flows from the risk estimation in Table 3 to the responses outlined in Table 4.

Risk level	Explanation and management response
1-2 Negligible	Acceptable level of risk. No immediate action required.
3-5 Low	Acceptable level of risk. On-going monitoring may be required.
6-10 Medium	Unacceptable level of risk. Active management is required to reduce the level of risk.
12-15 High	Unacceptable level of risk. Intervention is required to mitigate the level of risk.
16-25 Extreme	Unacceptable level of risk. Urgent intervention is required to mitigate the level of risk.

Table 22: Risk levels and management responses

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Water source (and entrained organisms in water) to farm/pond/tank	Surface waters that communicate with wild carp populations including areas connected only during flooding. Crustaceans, molluscs and planktons may potentially be carriers of CyHV-3 and transmit pathogens to koi.	Certain (5)	Catastrophic (5)	**Extreme (25)**
Introduction of new koi onto premise	High potential for some koi to be infected (sub-clinical carrier).	Likely (4)	Catastrophic (5)	**Extreme (20)**
Introduction disease from non-target species onto the facility/premise	Non-target species may become carriers of CyHV-3.	Possible (3)	Catastrophic (5)	**High (15)**
Introduction of disease from birds, vermin and pets/farm animals.	Regurgitated/digested CyHV-3 infected carp/koi may be infective. Birds, vermin and pet/farm animals may come into contact with CyHV-3 infected fish and/or contaminated material and bring infected material back to koi ponds/tanks.	Possible (3)	Catastrophic (5)	**High (15)**
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	People and equipment that have come into contact with water contaminated or fish infected with CyHV-3 may potentially be vectors for the disease	Possible (3)	Catastrophic (5)	**High (15)**
Introduction of disease (CyHV-3) through feed.	No feeding of raw fish. Only extruded pellets are fed. A slight potential that extruded feed could come into contact with a source of the virus.	Unlikely (2)	Catastrophic (5)	**Medium (10)**

B: Risks for pathogen (CyHV-3) spread within a koi facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Transmission of virus through aerosol/ water movement within a koi facility	Movement of the pathogen may occur via aerosol movement. Water which has not been treated may have come into contact with CyHV-3 infected fish which may allow for the virus to transmit to multiple tanks/ponds.	Likely (4)	Catastrophic (5)	**Extreme (20)**
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	People and equipment that have come into contact with water contaminated or fish infected with CyHV-3 may potentially be vectors for the disease. Equipment not dedicated to one fish group.	Possible (3)	Catastrophic (5)	**High (15)**
Movement of disease by birds, vermin, pets/farm animals between ponds/tanks	Diseased/latently infected fish are present on site.	Likely (4)	Catastrophic (5)	**Extreme (20)**
Spread of disease (CyHV-3) through feed.	Feed may potentially become contaminated with CyHV-3 and act as a vector for disease. Elevated opportunity for contamination of feed once virus is established on-site.	Possible (3)	Catastrophic (5)	**High (15)**

C: Risk to other koi facilities of release of CyHV-3 from an infected facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Discharge of pond water into storm water drainage	CyHV-3 may be present and viable in water from a pond containing infected koi (whether clinically, of sub-clinically infected).	Likely (4)	Catastrophic (5)	**Extreme (20)**
Discharge of pond water into wastewater (sewer) source	CyHV-3 may be present and viable in water from a pond containing infected koi (whether clinically, of sub-clinically infected).	Likely (4)	Insignificant (1)	**Low (4)**

Release of CyHV-3 through carriage of infected fish, or water which contains CyHV-3, by birds, vermin, pets/farm animals contacting infected site and moving to water bodies outside of the koi site.	Birds, vermin, pets/farm animals have contact/access with the infected pond water and fish population.	Possible (3)	Catastrophic (5)	**High (15)**
Disposal of mortalities	Fish mortalities are not promptly removed, buried, burnt, or bagged.	Possible (3)	Catastrophic (5)	**High (15)**
Release of the disease by people and equipment to wild riverine water source	People and equipment that have come into contact with water contaminated by, or fish infected with, CyHV-3 may potentially be vectors for the disease	Possible (3)	Catastrophic (5)	**High (15)**

D: Risks of pathogen (CyHV-3) spread at auctions and koi shows

Risk activity	**Assumption**	**Likelihood**	**Consequence**	**Risk rating**
Exposure through mixing of koi populations	Sub-clinically infected koi at shows/auctions will come into contact with uninfected koi. Purchasing of infected koi increases risk of spread of disease among existing stock at show, and upon return of stock to koi owner's site.	Certain (5)	Catastrophic (5)	**Extreme (25)**
Exposure to CyHV-3 through contaminated equipment and/or people	People and equipment will come into contact with water contaminated by, or fish infected with, CyHV-3.	Likely (4)	Catastrophic (5)	**Extreme (20)**

3. Legend

Shaded boxes:

Blue:	Suggestions made by industry stakeholder(s).
Green:	Agreed by industry stakeholder(s) to be a feasible option.
Orange:	Best option agreed by industry stakeholder(s), however with severe limitations inhibiting feasibility and/or adoptability by the industry.
Purple:	Most adoptable and feasible option agreed by industry stakeholder(s), however with severe risk of disease to the industry.
Red:	No agreed option by the industry stakeholder(s) due to severe limitations on feasibility and/or adoptability.

3.1 Text:

Red:	Changes made from stakeholder(s) comments and notes.
~~Crossout~~:	Agreed by stakeholder(s) to be not feasible and/or adoptable by the industry.

Likelihood reduction rating:

-0:	No change in likelihood rating or risk level
-1:	Mild reduction of likelihood rating (i.e Certain to Likely)
-2:	Reduction of likelihood rating (i.e Certain to Possible)
-3:	Moderate reduction of likelihood rating (i.e Certain to Unlikely)
-4:	Marked reduction of likelihood rating (i.e Certain to Remote)

3.2 Likelihood rating prior to applying reduction rating:

(1)	Remote
(2)	Unlikely
(3)	Possible
(4)	Likely
(5)	Certain

3.3 Risk rating:

Risk level	Explanation and management response
1-2 Negligible	Acceptable level of risk. No immediate action required.
3-5 Low	Acceptable level of risk. On-going monitoring may be required.
6-10 Medium	Unacceptable level of risk. Active management is required to reduce the level of risk.
12-15 High	Unacceptable level of risk. Intervention is required to mitigate the level of risk.
16-25 Extreme	Unacceptable level of risk. Urgent intervention is required to mitigate the level of risk.

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility				
Risk activity	#	Agreed approach	Suggestion made by FFVS	Stakeholder feasibility and adoption limitations
Water source (and organisms entrained within) to farm/pond/tank. Risk rating: Extreme (25)	1	None.	Bore-water is used wherever possible. Where the use of bore-water is not feasible, lake/river/small dam waters are used with disinfection/ decontamination protocols applied. Likelihood reduction: -3 (5)	Feasibility: The use of bore-water is feasible for the industry if it is available and dependable. The use of lake/river/small dam waters is currently used, however there is a severe limitation on cost of implementing a decontamination protocol. Businesses are unlikely to see any return on investment. Adoptability: Use of townwater is not feasible. Lake, river, and small dam waters and bore-water are currently used by the industry depending on availability and cost. Likelihood reduction: -0 (5)
Disinfection/deactivation of CyHV-3 in incoming water. Risk rating: Extreme (25)	2	None.	Hold all incoming surface water for 7 days. This includes a safety factor in case virus deactivation is delayed. Likelihood reduction: -3 (5)	Feasibility: It is feasible for the industry to perform UV disinfection, ozone disinfection, chemical disinfection. The industry stakeholder has noted that it is difficult to hold water with the absence of other aquatic organisms such as mosquitofish, eels, turtles, frogs. It is not feasible for the industry to heat and cool large volumes of water. Adoptability: The cost of the UV and ozone disinfection units and filtration units are prohibitive for the industry. The cost of chemical disinfection is prohibitive for the industry. To implement a disinfection protocol for incoming waters, significant investment must be made by the business which is unlikely to see any return on investment.

				Likelihood reduction: -0 (5)
~~Deactivation of CyHV-3 with bacteria isolates~~ Risk rating: Extreme (25)	~~3~~	~~None~~	Not feasible or adoptable Likelihood reduction: -0 (5)	Feasibility: Not available in Australia. Adoptability: Not adoptable, unlikely to yield any benefits in reducing risk, unlikely to be economically viable as an option. Likelihood reduction: -0 (5)
Management of water quality Risk rating: High (15)	4	Testing ~~and record~~ when required: Dissolved oxygen (DO), Temperature, pH, Salinity, Total Ammonia Nitrogen (TAN), algal blooms to maintain good water quality. Maintain clean ponds.		Feasibility: Feasible option. Adoptability: Water quality testing may not be routinely performed. Time, staffing availability, cost are some limiting factors (i.e cost of staffing and equipment). Likelihood reduction: -1 (4)
Prevention of potentially infected planktons, molluscs and crustaceans entry into ponds/tanks. Risk rating: High (15)	5	None.	Use bore-water as the water source. Likelihood reduction: -2 (3)	Feasibility: Use of town-water is not applicable. Use of borewater may be feasible. Adoptability: Fine filtration (down to 5 microns) likely to restrict flow on the farm. Cost of filtration, equipment and pumping likely to be a limiting factor for filtration. Bore-water quality may vary dramatically, and treatment of the water may be too expensive for businesses. Where the use of bore-water is feasible, would be the preferred option. The use of chemicals is cost prohibitive. The organisms are likely to re-establish soon after its usage.

			Likelihood reduction: -0 (3)
Prevention of wild carp entry into ponds/tanks. Risk rating: Low (5)	6 Ensure that adequate filtration is in place to ensure that no carp/eggs can enter the system through intake water.		Feasibility: Feasible to filter water to exclude carp entry. Adoptability: Fine filtration likely to restrict flow on the farm. Cost of filtration and pumping likely to be a limiting factor for this option. Likelihood reduction: -4 (5)
Risk rating: Medium (10)	Ensure adequate fencing/netting are in place to prevent bird access to ponds/tanks.		Feasibility: Feasible to place netting and fencing around ponds. It is impractical for farms to prevent small bird (i.e kingfisher) entry into ponds with netting. Netting and fencing will not keep turtles and frogs out of ponds. Adoptability: The stakeholder stated that many farms do have surface netting available. However, its use is limited when fish are small and are at higher risk of predation. The cost of netting is a limiting factor for farms. Netting also require constant maintenance for the farmers. Often wildlife can become entangled in netting especially after storms which requires time and money to release them from the nettings. Likelihood reduction: -2 (4)
Introduction of new koi onto premise	7 ~~Only source fish from specific pathogen free (SPF) (free of CyHV-3) certified sites/facilities where possible (none presently certified-require establishment if CyHV-3 released).~~	Not feasible or adoptable Likelihood reduction: -0 (4)	Feasibility: No SPF stock available in Australia.

Risk rating: Extreme (20)				Adoptability: Cost of obtaining SPF status is unlikely to yield any economic benefits for farms in Australia. Likelihood reduction -0 (4)
Risk rating: Negligible (1)		None	Do not stock / introduce any new broodstock or koi fish to the koi farm. Likelihood reduction -3 (4)	Feasibility: The stakeholder has not stocked/ introduced any new broodstock or koi fish to the farm since it first started farming koi. Access to diagnostic laboratories is limited for CyHV-3. Currently there are no vaccines available in Australia for CyHV-3. Adoptability: Cost of diagnostic tests likely to exceed the value of fish and make the business unprofitable. Sourcing fish from areas without a history of CyHV-3 poses significant risk as it relies on trust and people may not be aware of their fish's disease status. The efficacy of the vaccines is unlikely to yield any benefits to the business. Likelihood reduction: -0 (4)
Duration of quarantine period Risk rating: Extreme (20)	8a	None	Quarantine all new and returning fish upon arrival/return for a minimum of three (3) weeks, provides greater safety margin Likelihood reduction: -2 (4)	Feasibility: Feasible to hold fish in quarantine for 3 weeks. Adoptability: The stakeholder does not have a dedicated quarantine facility and have not introduced any new broodstock or koi to the farm. The cost of setting up a dedicated quarantine facility is considered financially unjustifiable. Likelihood reduction: -0 (4)

Cohabitation with sentinel koi Risk rating: Extreme (20)	8b	None	Quarantine all new and returning fish with thirty (30) sentinel koi for the duration of the quarantine period Likelihood reduction: -2 (4)	Feasibility: Stakeholder has never introduced new koi to founder population. There are currently no sentinel koi available in Australia. Adoptability: Cost of purchasing and maintaining 10-30 sentinel koi is uneconomic for the business. Likelihood reduction: -0 (4)
Quarantine temperature ranges Risk rating: Extreme (20)	8c	None	Quarantine all new and returning fish upon arrival/return at permissive temperature for KHVD (16 ^{o}C – 28 ^{o}C). Likelihood reduction: -2 (4)	Feasibility: It is feasible for the stakeholder to heat and cool water. Adoptability: The cost of equipment and electricity to heat and cool water and space required to maintain permissive temperatures for pathogen transmission and for KHVD is too costly. Likelihood reduction: -0 (4)
Monitoring of quarantine koi Risk rating: High (15)	8d	Observe quarantine fish daily ~~and record and document for~~ any abnormal observations ~~or test findings in a journal or an appropriate recording system~~.		Feasibility: It is feasible for the stakeholder to observe quarantine fish daily. Should new fish be introduced, it is likely they would receive prophylactic treatment with formalin, salt, Lepidex (Trichlorfon) and held for 1-3 days prior to introduction to the existing stock. Brief visual examination is likely to be performed daily with the existing stock. Adoptability: The time required to document findings is too time consuming for the stakeholder. The stakeholder has never introduced new koi to founder population.

				Likelihood reduction: -1 (4)
Selection of quarantine location Risk rating: Medium (10)	8e	Monitor all new and returning fish in a dedicated quarantine system for signs of disease.		Feasibility: Stakeholder has never introduced new koi to founder population was started. It is feasible for koi to be monitored in a dedicated quarantine system. Adoptability: The cost of setting up a dedicated quarantine facility is financially unjustifiable. . Likelihood reduction: -2 (4)
Additional quarantine barriers (dedicated equipment and disinfection of equipment) Risk rating: Low (5)	8f	All quarantine equipment remains in quarantine area.		Feasibility: Feasible to keep dedicated quarantine equipment on site. Adoptability: Cost of purchasing dedicated quarantine equipment. Likelihood reduction: -3 (4)
Risk rating: Low (5)	8g	Where no alternative exists, and the use of non-dedicated equipment is required, non-dedicated equipment should be disinfected with chemicals described in Table 6 prior to use.		Feasibility: Feasible to use disinfectants. Adoptability: Storage space required to store chemicals if large quantities are required. Likelihood reduction: -3 (4)
Deliberate stressors during quarantine	8h	None	Apply transport, temperature, handling and sampling stress to new and/or sentinel koi during the quarantine period.	Feasibility: It is feasible for the stakeholder to apply stressors to the new fish.

Risk rating: Extreme (20)			Likelihood reduction: -2 (4)	Adoptability: The cost of setting up a quarantine facility and for diagnostic tests is uneconomic for the stakeholder. The space required to hold fish is limited on the stakeholder's farm. Likelihood reduction: -0 (4)
Disease investigation/surveillance of sick/ freshly dead quarantined fish Risk rating: Extreme (20)	9	None	Sample all new fish non-lethally (mucus, blood and/or gill swab samples), and all sentinel koi lethally, at the end of the quarantine period by (kidney , spleen, gills) and perform at least three (3) molecular diagnostic tests (i.e. nested PCR, real time PCR, qPCR) for CyHV-3 and histology. Likelihood reduction: -2 (4)	Feasibility: Limited diagnostic laboratories available in Australia for CyHV-3. Diagnostic tests do not provide 100% exclusion. Currently, the stakeholder performs routine gill and skin mucous sampling. These tests do not provide any diagnostic exclusion for CyHV-3 and only aids in identification of ectopatasites. Contact with veterinarians is infrequent and only sort after significant disease has occurred and the stakeholder is unable to identify a plausible causation factor. Adoptability: Cost of diagnostic tests is unviable for the business. Likelihood reduction: -0 (4)
Health status of new fish Risk rating: Extreme (20)	10	Ensure that the health status of all new fish is adequate before stocking with existing stock.	Ensure health status is equal to, or higher, than the existing stock through: targeted testing (eg CyHV-3 PCR); selection from populations that have not exhibited disease outbreaks; appropriate import/translocation permits in place for stock movement. New fish are only to be introduced into the facility once all diagnostic test results have been received and are negative for CyHV-3. Likelihood reduction: -2 (4)	Feasibility: Stakeholder has never introduced new koi to founder population. Limited diagnostic laboratories available in Australia for CyHV-3. Diagnostic tests do not provide 100% exclusion. Adoptability: Concerns were raised regarding the cost of diagnostic tests if stocking was required. Fish are likely to be stocked if they look of sufficient health status if the cost of diagnostic tests are too high. Likelihood reduction: -0 (4)

Reduction of risk associated with cohabitation/stocking of potential non-target species Risk rating: Medium (10)	11 Do not cohabitate potential non-target species which been described to potentially harbour the virus with koi.		Feasibility: Stakeholder stocks silver perch and goldfish on site. Cohabitation is not performed in the grow out ponds, however, it is common for goldfish and koi to be cohabitated together in display tanks. The airspace between ponds are shared. No disinfection protocols are in place for recirculated waters. Adoptability: The option of removal of multi-species from some farms is not feasible due to economic impact of lost species and inability to operate biosecure separation cost-effectively. Likelihood rating: -1 (3)
Reduction of access of vermin (i.e. birds, rodent) and pets/farm animals to koi ponds/ tanks/ associated infrastructure. Risk rating: Low (5)	12 Erect bird fences to eliminate birds/pets from entering ponds. Control rodents by good feed storage and bait stations.		Feasibility: It is difficult to exclude all animals from entering the ponds even with netting and fencing. Farm animals such as dogs and cattle require substantial fences, not presently in place, to reliably exclude from pond access. Many farms have bird netting in place. However, it is impractical for keeping small birds such as kingfishers out of ponds. Netting may not be used at all times as wildlife often become entangled in netting especially after storms which requires time to release them from the netting. Netting and fencing will not keep turtles and frogs out of ponds. Adoptability: Time required to maintain netting and fencing and the cost to replace netting and fencing are uneconomic for the business Likelihood rating: -2 (3)
Minimisation of contact with potentially contaminated equipment	13 Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should not be brought into the facility.	*Note: It is to FFVS's belief that the cost of efficacious disinfectants are unlikely to be too*	Feasibility: It is possible for dedicated equipment to be used on site. Stakeholder shares equipment throughout the facility. Used equipment are sun dried after each use. If the equipment is dirty, then they are cleaned with

Risk rating: Low (5)		Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry.	*expensive for the industry, or require substantial equipment.*	detergent prior to being sun dried. All equipment are disinfected with hypochlorite at the end of the season. Adoptability: Stakeholder raised concerns regarding the cost of chemicals and dedicated equipment. Concerns were raised regarding buyers and contractors. It is difficult to enforce buyers and contractors to disinfect their equipment and disinfection is unlikely to occur. It is believed that enforcing these rules will only result in loss of sales. Likelihood rating: -2 (3)
Minimisation of risks associated with people/visitors/staff that may have been exposed to potentially contaminated water or diseased fish. Risk rating: Medium (10)	14	Disinfect hands and footwear upon entry (and exit) to a koi facility, and between separate areas of facility Staff~~/visitors~~/owners must wear freshly laundered clothes each day prior to entry into a koi facility. All visitors/contractors/researchers must be aware of the biosecurity required prior to being granted entry. Visitors must be accompanied by a staff member/owner at all times Visitor vehicles to be parked in dedicated parking area, preferably remote to fish stock		Feasibility: It is feasible for the stakeholder to implement disinfection protocols for hands and footwear upon entry and exit to the farm. It is not feasible for the stakeholder to ensure that visitors are in freshly laundered clothes prior to entry to the farm. The stakeholder believes that a visitor logbook will be a deterrent for buyers and contractors and will be ineffective as they may not declare the truth. Visitors are accompanied by the stakeholder or associates when visiting. It may be difficult for the stakeholder to prevent access of non-farm vehicles to the ponds as his buyers prefer to load fish into their vehicles pond side. It is impractical to discourage visitors to touch production fish and associated waters and infrastructure. Adoptability: It is difficult to change human behaviour. The stakeholder believes that implementing all of the measures will discourage his buyers from purchasing fish from him and will go elsewhere or seek the black-market for fish. Likelihood rating: -1 (3)

		and fish movement loading area where possible.		
Avoidance of feeding potentially contaminated food. Risk rating: Negligible (1)	15	Feed sanitised food (i.e extruded pellets) only. Do not feed materials that has been potential contaminated with CyHV-3. Do not feed raw fish to koi.		Feasibility: Feasible to feed extruded pellets only. Currently being performed by the stakeholder. Adoptability: Easily adoptable. Likelihood rating: -1 (2)

B: Risks for pathogen (CyHV-3) spread within a koi facility

B: Risks for pathogen (CyHV-3) spread within a koi facility				
Risk activity	#	Agreed approach	Suggestion made by FFVS	Stakeholder feasibility and adoption limitations
Management of fish movement within a facility. Risk rating: Extreme (20)	1	None	Record all movement of all fish on site, between areas Likelihood reduction: -2 (4)	Feasibility: Not feasible for the stakeholder due to time required to perform task. Adoptability: The stakeholder noted that record keeping of all fish movement on site, between areas is time consuming and believes that there is very little financial reward. Likelihood reduction: -0 (4)
Management of water between systems. Risk rating: Low (5)	2	Where water is recirculated, ensure that appropriate measures (i.e with UV and/or ozone) are made to disinfect water to ensure that only sanitary water is recirculated		Feasibility: Option is viable. Adoptability: The cost of installing and maintaining disinfection measures on recirculated water will be too costly for most business to voluntarily uptake this measure. Likelihood reduction: -3 (4)
Reduction of aerosol spread between ponds/tanks. Risk rating: Extreme (20)	3	~~Ensure that all doors between facilities/sites remain closed at all times where possible.~~	Not feasible or adoptable Likelihood reduction: -0 (4)	Feasibility: Koi ponds are outdoor structures. Prevention of aerosols is impossible. Possible to prevent aerosol between zones (i.e quarantine if available). Adoptability: Not adoptable.

				Likelihood reduction: -0 (4)
Minimisation of contact with potentially contaminated equipment Risk rating: High (15)	4	Access to quarantine zones is avoided where possible		Feasibility: It is feasible to avoid access to the quarantine area where possible. Adoptability: The stakeholder raised concerns of the cost of purchasing multiple sets of equipment, some of which is expensive such as water quality meters. May be difficult for small farms with few staff members to allocate single staff member to perform biosecurity duties. It is possible for people to reduce access to quarantine areas where possible. Likelihood reduction: -1 (3)
Detection of latently infected fish Risk rating: Extreme (20)	5	None	Routinely sample skin mucus and gill mucus every six months/year and perform 2 PCR methods For ELISA tests, at least ten blood samples from separate individual koi are required. Apply stressors including transport, handling, temperature fluctuations and sampling stress 3-6 days prior to collection of samples. Likelihood reduction: -2 (4)	Feasibility: The sensitivity of the diagnostic tests do not provide 100% exclusion of the pathogen (CyHV-3). Access to diagnostic laboratories is limited for CyHV-3. Adoptability: The cost of the diagnostic test is likely to make the stakeholder's business unviable. The stakeholder believes that he would not be able to see return on the investment as he is unable to reflect the cost back onto his customers. Likelihood reduction: -0 (4)
~~Vaccination of existing stock~~ Risk rating:	~~6~~	~~None~~	Not feasible or adoptable Likelihood reduction: -0 (4)	Feasibility: No vaccines available in Australia. Efficacy of vaccines is poor.

Extreme (20)				Adoptability: The cost of vaccines and time required to individually inject each fish will be too exorbitant on the stakeholder's business. The business will not see any benefit on investment as market is unwilling to accept price increases. Likelihood reduction: -0 (4)
Prevention of spread of disease between systems/site and to another systems/site Risk rating: Extreme (20)	7	In the event of a material disease in a pond/system, ~~all~~ affected fish populations are culled. A decontamination process (i.e. hydrated lime (CaOH) application and drying of ponds in sunlight for a minimum of 7 days) must be undertaken prior to restocking. Decontamination must be undertaken in consultation with, or under instruction from, the relevant state government biosecurity agency to ensure compliance with biosecurity obligations or with a competent veterinarian. Fish movement is restricted for a minimum of one year (or until water temperatures fall below permissible ranges the following year). No new fish are permitted to be introduced into the site/fishery during the restriction period.		Feasibility: Culling of fish will likely be performed for affected populations (affected ponds/tanks only) only. Adoptability: Culling will likely result in the cessation of farming due to the loss of genetic lines. These lines are difficult to re-establish, and at times impossible due to availability in Australia. Likelihood reduction: -1 (5)

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility				
Risk activity	#	Agreed approach	Suggestion	Stakeholder feasibility and adoption limitations
Management of effluent discharge Risk rating: Extreme (20)	1	None	Hold all untreated/filtered effluent water for 7 days prior to discharge. This includes a safety factor in case virus deactivation is delayed. Likelihood reduction: -3 (4)	Feasibility: Most water is recirculated by the stakeholder. It is not feasible for the stakeholder to treat all effluent water with large quantities of disinfectants. Adoptability: There are difficulties for the stakeholder to hold water without any other aquatic creatures such as mosquitofish, eels, frogs, turtles. The cost of large quantities of disinfectants is uneconomic for the stakeholder. Likelihood reduction: -0 (4)
Appropriate disposal of mortalities Risk rating: Medium (10)	2	Fish mortalities are ~~recorded and~~ removed daily and disposed of by a method approved by the relevant authority, which ensures no risk of release of pathogens from the dead stock into waterways, or access for scavenger birds or animals (e.g. pigs, foxes, water rats) that could spread a disease.		Feasibility: The stakeholder disposes all mortalities by deep burial on site. No record keeping performed. Adoptability: Currently performed by the stakeholder – easily adoptable. Likelihood reduction: -1 (3)
Management of people Risk rating:	3	None	Disinfect hands and footwear upon exiting the farm/site.	Feasibility: Disinfection of upon exit of the facility is possible, however it would likely be a deterrent for buyers and contractors which will go elsewhere to purchase fish.

High (15)			Likelihood reduction: -2 (3)	Adoptability: It would be unlikely that this would be adopted. The stakeholder believes that he would have a significant loss in revenue by implementing disinfection protocols for people on entry and exit. Likelihood reduction: -0 (3)
Management of equipment Risk rating: Medium (10)	4	Dedicated equipment should be ~~labelled and~~ maintained for use exclusively on site. Dedicated equipment should not be removed from site and use for other purposes.		Feasibility: Stakeholder keeps dedicated equipment on the farm. No routine labelling is performed. Adoptability: Labelling is unlikely to be performed. Likelihood reduction: -1 (3)

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows

Not Applicable.

Appendix 1: Table 6 – Disinfection dosages against CyHV-3

Disinfectant	Treatment time	Temperature (15°C)	Temperature (25°C)	Temperature (unspecified)
Iodophor (mg/L)	30 s	200	200	N/A
	20 min	200	200	N/A
Sodium hypochlorite solution (mg/L)	30 s	>400	>400	N/A
	20 min	200	250	N/A
Benzalkonium chloride solution (mg/L)	30 s	60	30	N/A
	20 min	60	30	N/A
Ethyl alcohol (%)	30 s	40	30	N/A
	20 min	30	25	N/A
Free Chlorine (mg/L)	30 min	N/A	N/A	3

Table 23 Chemicals used for disinfection of CyHV-3 adapted from Kasai et al. 2005.

Appendix 6 – Phase 3 Hobbyist options

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Feasibility of Adoption of Biosecurity and Control strategies against Cyprinid Herpesvirus 3 (CyHV-3) for koi industry hobbyists in Australia in relation to the potential release of the virus as a biocontrol agent.

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20 June 2019

Contents

1. Introduction

Cyprinid Herpesvirus 3 (CyHV-3) also known as Koi Herpesvirus (KHV) is a notifiable disease in koi (*Cyprinus carpio koi*) and European carp (*Cyprinus carpio*). CyHV-3 is the pathogen responsible for causing Koi Herpesvirus Disease (KHVD) which is the damaged tissues which result from viral replication. In subclinical infections with CyHV-3, no tissue damage may be evident, hence the fish is not technically diseased. It is however an infected carrier of the virus which could potentially spread to other susceptible fish. In recent years, CyHV-3 has been explored as a possible biocontrol measure for European carp in Australia. This virus has been detected in 33 countries including Canada, Indonesia, Japan, Taiwan, Thailand, Poland, Singapore, United Kingdom, and the United States of America.

The potential release of CyHV-3 into Australian waters would introduce new risks to Australia's koi industry. A literature review and biosecurity options list were compiled to assess and explore potential risk mitigation strategies against CyHV-3. A stakeholder meeting in Sydney was held to explore each biosecurity options identified from the literature review.

This report draws from the previous biosecurity options report circulated prior to the meeting. Agreed approaches by industry stakeholder(s) are placed in the column titled "Agreed approach". Where "None" is written, no agreed option from biosecurity options paper was reached by the stakeholder(s). The feasibility, adoptability and likelihood reduction rating of each option are explored in the "Stakeholder feasibility and adoption limitations" column.

The likelihood reduction rating assesses the level of reduction in likelihood of the risk activity after applying the biosecurity option listed in the "Agreed approach" column. Where no agreed approach was reached, the likelihood reduction rating is listed in the suggestion column. Suggestions made by FFVS have taken the feasibility and adoptability of each biosecurity options into account. The overall risk rating after applying the agreed approach or the stakeholder(s) suggested approach to the risk is listed in the "Risk activity" column.

2. Biosecurity Risk Analysis of CyHV-3

This process helps identify the areas which require the greatest biosecurity investment to deliver maximum protection to the farm, or hobbyist pond and tanks, from incursion of CyHV-3 and disease impacts of Koi Herpesvirus Disease (KHVD).

Figure 4: Four steps of biosecurity risk analysis

2.1 Identify the hazards

In this project, the hazard being assessed is CyHV-3. Other hazards are outside the scope of the project, but can be considered using this framework by koi owners on another occasion.

CyHV-3 has been identified as a hazard because it has caused adverse impacts on fish health and production internationally. Additionally, this disease has not occurred in Australia (presently considered exotic), and should it be released as part of a carp control program, koi owners' / farmers' stock will be placed at an increased risk.

2.2 Risk Assessment of hazards

To assign a level of risk to a hazard, two factors need to be determined – the likelihood of exposure on your site and the consequence(s) of it occurring on your site. Veterinarians with an interest in aquatic species will be able to assist with this section.

Likelihood can be estimated by considering the transmission pathways necessary for entry of a pathogen (disease causing agent-CyHV-3), and for exposure of your fish. For example, should CyHV-3 be released into the wild, the likelihood of exposure via water, when using water sources that contain wild carp will be 'certain', if the carp control program seeks to use the virus to control carp in all wild waters. If your facility is using chlorinated town water as the source, then the entry of CyHV-3 via this route, would be considered 'remote' as chlorination would be expected to deactivate the virus.

Similarly, pathways involving entry of infected (either clinical (expressing signs of disease) or sub-clinical (not exhibiting any obvious external signs of sickness)) live fish have the highest likelihood of causing exposure because they may shed the pathogen into your naïve, clean koi population.

The likelihood rating for exposure will vary depending on:

- the properties of the pathogen
- the occurrence of the pathogen outside the site or in nearby sites and
- the possible pathways onto the site

Likelihood ratings and descriptors are shown in Table 1.

Rating	Descriptor
Remote (1)	Occurs less than once in 20 years
Unlikely (2)	Occurs not more than once in 5-20 years
Possible (3)	Occurs not more than once in 3-5 years
Likely (4)	Occurs not more than once in 2 years
Certain (5)	Occurs every year

Table 24: Assessment of disease likelihood

Consequence can be estimated by considering the impact(s) of the disease (where the pathogen has damaged tissues of the host fish) on the productivity/health of your fish population and enterprise. The consequences could include multiple aspects (e.g. mortality, reduced growth or food conversion, reduced product quality, reduced market access, lost sales, emotional stress and trauma from loss of a pet, and/or treatment costs).

Consequence ratings and descriptors are shown in Table 2.

Rating	Descriptor
Insignificant (1)	Impact not detectable or minimal
Minor (2)	Impact is limited to some, not all, units and/or short term only
Moderate (3)	Impact of most populations on site, with increased mortality and/or decreased performance, but not business or hobby ending. Stock loss may result in some emotional stress.
Major (4)	All populations affected. Considerable impact resulting in serious supply constraints, stock loss and financial impact, some emotional trauma.
Catastrophic (5)	All populations affected. Likely complete depopulation of the site and possibly barriers to resumption of production/hobby, highly significant emotional trauma.

Table 25: Assessment of disease consequences

Risk estimation—Risk is estimated as a product of likelihood and consequence, resulting in risk ratings of 1–25. Risks are highest when both likelihood and consequence are high. However, the risk may be low even if the consequence is 'catastrophic', as the likelihood may be 'remote' for that particular circumstance; similarly, even if the likelihood is 'certain', the consequence may be 'insignificant'. Risk ratings can be determined by applying estimates of likelihood (where 1 is remote and 5 is certain) and consequence (where 1 is insignificant and 5 is catastrophic) to the risk matrix provided below in Table 3.

Likelihood rating / Consequence rating	Insignificant	Minor	Moderate	Major	Catastrophic
Remote	1	2	3	4	5
Unlikely	2	4	6	8	10
Possible	3	6	9	12	15
Likely	4	8	12	16	20
Certain	5	10	15	20	25

Table 26: Risk estimation matrix

The need for risk mitigation management responses flows from the risk estimation in Table 3 to the responses outlined in Table 4.

Risk level	Explanation and management response
1-2 Negligible	Acceptable level of risk. No immediate action required.
3-5 Low	Acceptable level of risk. On-going monitoring may be required.
6-10 Medium	Unacceptable level of risk. Active management is required to reduce the level of risk.
12-15 High	Unacceptable level of risk. Intervention is required to mitigate the level of risk.
16-25 Extreme	Unacceptable level of risk. Urgent intervention is required to mitigate the level of risk.

Table 27: Risk levels and management responses

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Water source (and entrained organisms in water) to farm/pond/tank	Surface waters that communicate with wild carp populations including areas connected only during flooding. Crustaceans, molluscs and planktons may potentially be carriers of CyHV-3 and transmit pathogens to koi.	Certain (5)	Catastrophic (5)	**Extreme (25)**
Introduction of new koi onto premise	High potential for some koi to be infected (sub-clinical carrier).	Likely (4)	Catastrophic (5)	**Extreme (20)**
Introduction disease from non-target species onto the facility/premise	Non-target species may become carriers of CyHV-3.	Possible (3)	Catastrophic (5)	**High (15)**
Introduction of disease from birds, vermin and pets/farm animals.	Regurgitated/digested CyHV-3 infected carp/koi may be infective. Birds, vermin and pet/farm animals may come into contact with CyHV-3 infected fish and/or contaminated material and bring infected material back to koi ponds/tanks.	Possible (3)	Catastrophic (5)	**High (15)**
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	People and equipment that have come into contact with water contaminated or fish infected with CyHV-3 may potentially be vectors for the disease	Possible (3)	Catastrophic (5)	**High (15)**
Introduction of disease (CyHV-3) through feed.	No feeding of raw fish. Only extruded pellets are fed. A slight potential that extruded feed could come into contact with a source of the virus.	Unlikely (2)	Catastrophic (5)	**Medium (10)**

B: Risks for pathogen (CyHV-3) spread within a koi facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Transmission of virus through aerosol/ water movement within a koi facility	Movement of the pathogen may occur via aerosol movement. Water which has not been treated may have come into contact with CyHV-3 infected fish which may allow for the virus to transmit to multiple tanks/ponds.	Likely (4)	Catastrophic (5)	**Extreme (20)**
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	People and equipment that have come into contact with water contaminated or fish infected with CyHV-3 may potentially be vectors for the disease. Equipment not dedicated to one fish group.	Possible (3)	Catastrophic (5)	**High (15)**
Movement of disease by birds, vermin, pets/farm animals between ponds/tanks	Diseased/latently infected fish are present on site.	Likely (4)	Catastrophic (5)	**Extreme (20)**
Spread of disease (CyHV-3) through feed.	Feed may potentially become contaminated with CyHV-3 and act as a vector for disease. Elevated opportunity for contamination of feed once virus is established on-site.	Possible (3)	Catastrophic (5)	**High (15)**

C: Risk to other koi facilities of release of CyHV-3 from an infected facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Discharge of pond water into storm water drainage	CyHV-3 may be present and viable in water from a pond containing infected koi (whether clinically, of sub-clinically infected).	Likely (4)	Catastrophic (5)	**Extreme (20)**
Discharge of pond water into wastewater (sewer) source	CyHV-3 may be present and viable in water from a pond containing infected koi (whether clinically, of sub-clinically infected).	Likely (4)	Insignificant (1)	**Low (4)**

Release of CyHV-3 through carriage of infected fish, or water which contains CyHV-3, by birds, vermin, pets/farm animals contacting infected site and moving to water bodies outside of the koi site.	Birds, vermin, pets/farm animals have contact/access with the infected pond water and fish population.	Possible (3)	Catastrophic (5)	**High (15)**
Disposal of mortalities	Fish mortalities are not promptly removed, buried, burnt, or bagged.	Possible (3)	Catastrophic (5)	**High (15)**
Release of the disease by people and equipment to wild riverine water source	People and equipment that have come into contact with water contaminated by, or fish infected with, CyHV-3 may potentially be vectors for the disease	Possible (3)	Catastrophic (5)	**High (15)**

D: Risks of pathogen (CyHV-3) spread at auctions and koi shows

Risk activity	**Assumption**	**Likelihood**	**Consequence**	**Risk rating**
Exposure through mixing of koi populations	Sub-clinically infected koi at shows/auctions will come into contact with uninfected koi. Purchasing of infected koi increases risk of spread of disease among existing stock at show, and upon return of stock to koi owner's site.	Certain (5)	Catastrophic (5)	**Extreme (25)**
Exposure to CyHV-3 through contaminated equipment and/or people	People and equipment will come into contact with water contaminated by, or fish infected with, CyHV-3.	Likely (4)	Catastrophic (5)	**Extreme (20)**

3. Legend

Shaded boxes:

Blue:	Suggestions made by industry stakeholder(s).
Green:	Agreed by industry stakeholder(s) to be a feasible option.
Orange:	Best option agreed by industry stakeholder(s), however with severe limitations inhibiting feasibility and/or adoptability by the industry.
Purple:	Most adoptable and feasible option agreed by industry stakeholder(s), however with severe risk of disease to the industry.
Red:	No agreed option by the industry stakeholder(s) due to severe limitations on feasibility and/or adoptability.

3.1 Text:

Red: Changes made from stakeholder(s) comments and notes.
~~Crossout~~: Agreed by stakeholder(s) to be not feasible and/or adoptable by the industry.

Likelihood reduction rating:

-0: No change in likelihood rating or risk level
-1: Mild reduction of likelihood rating (i.e Certain to Likely)
-2: Reduction of likelihood rating (i.e Certain to Possible)
-3: Moderate reduction of likelihood rating (i.e Certain to Unlikely)
-4: Marked reduction of likelihood rating (i.e Certain to Remote)

3.2 Likelihood rating prior to applying reduction rating:

(1) Remote
(2) Unlikely
(3) Possible
(4) Likely
(5) Certain

3.3 Risk rating:

Risk level	Explanation and management response
1-2 Negligible	Acceptable level of risk. No immediate action required.
3-5 Low	Acceptable level of risk. On-going monitoring may be required.
6-10 Medium	Unacceptable level of risk. Active management is required to reduce the level of risk.
12-15 High	Unacceptable level of risk. Intervention is required to mitigate the level of risk.
16-25 Extreme	Unacceptable level of risk. Urgent intervention is required to mitigate the level of risk.

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility				
Risk activity	#	Agreed approach	Suggestion made by FFVS	Stakeholder feasibility and adoption limitations
Water source (and organisms entrained within) to farm/pond/tank. Risk rating: Low (5)	1	Town-water is used wherever possible. For rural areas with limited access to town-water, bore-water is used instead.		Feasibility: Town-water is used by most koi keepers. For people in rural areas, access to town-water may be limited. Adoptability: Easily adoptable for urban areas. For rural areas, access may be limited. Likelihood reduction: -4 (5)
Disinfection/deactivation of CyHV-3 in incoming water. Risk rating: Low (5)	2	Disinfect all incoming water with UV light at 4.0×10^3 µWs/cm^2.		Feasibility: Feasible for UV (medical) light to be used as a disinfectant. The level of understanding and knowledge to safely maintain and keep an ozone unit is beyond many keepers. Adoptability: Cost of UV likely out of reach of some hobbyists. The space required to hold large volumes of water is prohibitive. Likelihood reduction: -4 (5)
~~Deactivation of CyHV-3 with bacteria isolates~~ Risk rating: Extreme (25)	~~3~~	~~None~~		Feasibility: Not feasible. There is uncertainty around the efficacy using bacteria to deactivate CyHV-3 Adoptability: Not adoptable. Cost likely prohibitive. Difficult to hold large volumes of water for 3-7 days.

			Likelihood reduction: -0 (5)
Management of water quality Risk rating: High (15)	4 Testing and record: ~~Dissolved oxygen (DO),~~ Temperature, pH, ~~Salinity,~~ Total Ammonia Nitrogen (TAN), nitrite, nitrate, algal blooms (visually) to maintain good water quality. Maintain clean ponds.		Feasibility: Water quality measuring is feasible. Most koi keepers do not perform water quality tests routinely (daily). It may be difficult to reach non-koi club keepers which will continue to pose a significant risk to the industry through uncontrolled fish movements, if stringent biosecurity practices are not employed. Adoptability: Measuring of temperature, ammonia, nitrite, nitrate, pH could be performed. Other tests likely to be too expensive or beyond the expertise of a normal koi keeper. Education would be required to increase adoption of the option and to change behaviours of keepers. Likelihood reduction: -1 (4)
Prevention of potentially infected planktons, molluscs and crustaceans entry into ponds/tanks. Risk rating: Low (5)	5 Use bore-water or town-water as the water source	*Note: It is to FFVS's belief that the cost of efficacious disinfectants are unlikely to be too expensive for the industry, or require substantial equipment.*	Feasibility: Filtration of surface riverine water down to 5 microns is not feasible for the industry. The use of bore-water or town-water is the most technically feasible and readily adoptable option for the industry. Adoptability: The cost of chemical usage is likely to be cost prohibitive for the industry to treat unsanitary water sources such as riverine water. Likelihood reduction: -3 (4)
Prevention of wild carp entry into ponds/tanks.	6 None	Use of carp free water sources (bore-water or town-water) as the water source	Feasibility: Fine filtration is not feasible for the industry. The use of water sources that are free of carp is the most viable option for the industry.

Risk rating: Low (5)			Likelihood reduction: -4 (5)	Adoptability: Fine filtration is not possible for the industry. The use of town-water or bore-water can be adoptable by the industry. Likelihood reduction: -4 (5)
Risk rating: Extreme (20)		None	Ensure adequate fencing/netting are in place to prevent bird access to ponds/tanks. Likelihood reduction: -2 (4)	Feasibility: It may be feasible to erect nets/fences to exclude bird access to ponds. However, aesthetically is unappealing and for some it defeats the purpose of having a koi pond Adoptability: Ongoing cost of management and replacement (likely every 5 years) is prohibitive. Birds may still get in and pose a risk. Likelihood reduction: -0 (4)
Introduction of new koi onto premise Risk rating: High (15)	7	Only source fish from disease free facilities with known histories.	Use of diagnostics to exclude diseases such as CyHV-3. Likelihood reduction: -2 (4)	Feasibility: Unlikely to be effective. Previous outbreaks of Aermonas spp bacteria have highlighted that people will still sell infected fish and that there is low confidence in people providing a sufficiently reliable history. Adoptability: Stakeholders held concerns of dissemination by carrier fish being likely with adoption of this low level control method. Likelihood reduction: -1 (4)
Duration of quarantine period Risk rating:	8a	None	Quarantine all new and returning fish upon arrival/return for a minimum of three (3) weeks.	Feasibility: This is not feasible for many koi keepers. Many koi keepers do not have adequate space or facilities to hold fish in quarantine for extended periods.

Extreme (20)			Likelihood reduction: -2 (4)	Adoptability: Cost likely to be prohibitive. Some larger koi keepers do have separate tanks that they may use as quarantine tanks. However, the aerosol space is still shared. The cost in installing a dedicated quarantine building suggests the prospects of widespread adoption are remote. Likelihood reduction: -0 (4)
~~Cohabitation with sentinel koi~~ Risk rating: Extreme (20)	~~8b~~	~~None~~		Feasibility: Not feasible for many koi keepers. Many koi keepers will not have a dedicated quarantine facility. A significant sized system would be required to hold the target koi and thirty sentinel koi Adoptability: The cost and space required is prohibitive. Thirty koi is at times more koi than a keeper would have in their collection. Ten sentinel koi may be more adoption by the industry but would require the keeper to have access to a dedicate quarantine facility big enough to hold the fish. Concerns were raised by the stakeholders regarding the diagnostic sensitivity of testing less fish. Likelihood reduction: -0 (4)
Quarantine temperature ranges Risk rating: Extreme (20)	8c	None	Quarantine all new and returning fish upon arrival/return at permissive temperature for KHVD (16 ^{o}C – 28 ^{o}C). Likelihood reduction: -2 (4)	Feasibility: Given the lack of capacity to install quarantine facilities, this measure is deemed not feasible. Adoptability: The further costs to establish equipment to heat or cool water to establish permissive ranges are further barriers to adoption. Likelihood reduction: -0 (4)
Monitoring of quarantine koi	8d	Observe quarantine fish daily ~~and record and document~~ for any abnormal observations ~~or~~		Feasibility: Most hobbyist will have a full time job which will restrict the ability for the keepers to closely monitor their

Risk rating: Extreme (20)		~~test findings in a journal or an appropriate recording system~~.		quarantine fish. Record keeping occurs only where time permits. Given the lack of capacity to install quarantine facilities, this measure is deemed not feasible. Adoptability: Keepers are unlikely to recognise diseases or clinical signs unless trained. Education would be required. Non-koi club keepers will pose a significant risk as access to information and training may be limited. Monitoring and documentation are required to support proper quarantine. Likelihood reduction: -0 (4)
Selection of quarantine location Risk rating: Extreme (20)	8e	Monitor all new and returning fish in a dedicated quarantine system for signs of disease.		Feasibility: Given the lack of capacity to install quarantine facilities, this measure is deemed not feasible. Adoptability: The space and cost associate with setting up a dedicated quarantine space is prohibitive for most keepers. Most of the public will not have access to a dedicated quarantine facility. Currently, new fish are stocked into existing ponds, and disease consequences are visually monitored. Likelihood reduction: -0 (4)
Additional quarantine barriers (dedicated equipment and disinfection of equipment) Risk rating: Extreme (20)	8f	All quarantine equipment remains in quarantine area.		Feasibility: Given the lack of capacity to install quarantine facilities, this measure is deemed not feasible. Adoptability: It was highly recommended by stakeholders for koi keepers to have dedicated equipment, however many koi keepers will not be able to afford two set of each equipment (i.e. nets, bowls, pumps).

				Likelihood reduction: -0 (4)
Risk rating: Extreme (20)	8g	Where no alternative exists, and the use of non-dedicated equipment is required, non-dedicated equipment should be disinfected with chemicals described in Table 6 prior to use.		Feasibility: Disinfection of equipment likely to be undertaken and is currently used by many keepers. Given the lack of capacity to install quarantine facilities, this measure is deemed not feasible. Adoptability: Adoption are limited by education of the utility of these techniques and the cost and human risk from handling potentially hazardous chemicals. Likelihood reduction: -0 (4)
Deliberate stressors during quarantine Risk rating: Extreme (20)	8h	None	Apply transport, temperature, handling and sampling stress to new and/or sentinel koi during the quarantine period. Likelihood reduction: -2 (4)	Feasibility: Some hobbyists do elicit stressors to fish during quarantine. Adoptability: Some hobbyists do elicit stressors to fish during quarantine. However, the lack of a quarantine facilities for many koi owners means this this option cannot be adopted. Likelihood reduction: -0 (4)
Disease investigation/surveillance of sick/ freshly dead quarantined fish Risk rating: Extreme (20)	9	None	Sample all new and returning fish non-lethally (mucus, blood and/or gill swab samples), and all sentinel koi lethally, at the end of the quarantine period by (kidney , spleen, gills) and perform at least three (3) molecular diagnostic tests (i.e. nested PCR, real time PCR, qPCR) for CyHV-3 and histology. Likelihood reduction: -2 (4)	Feasibility: Limited diagnostic laboratories available in Australia for CyHV-3. Diagnostic tests do not provide 100% exclusion. Diseases are rarely investigated – trial and error is elicited for many scenarios. Adoptability: It is not adoptable for many hobbyists due to the cost of diagnostic tests and the lack of facilities to run sentinel fish trials in.

				Likelihood reduction: -0 (4)
Health status of new fish Risk rating: Extreme (20)	10	Ensure that the health status of all new fish is adequate before stocking with existing stock.	Ensure health status is equal to, or higher, than the existing stock population through: targeted testing for relevant pathogens (eg CyHV-3); selection from populations that have not exhibited disease outbreaks; appropriate import/translocation permits in place for stock movement. New fish are only to be introduced into the facility once all diagnostic test results have been received and are negative for CyHV-3. Likelihood reduction: -2 (4)	Feasibility: Technically feasible Adoptability: Adoption is likely limited by the cost of diagnostic tests. The low diagnostic sensitivity of tests will limit the efficacy of this measure when used alone. Many koi keepers outside of clubs are unlikely to be aware of the existence and utility of tests. Likelihood reduction: -0 (4)
Reduction of risk associated with cohabitation/stocking of potential non-target species Risk rating: High (15)	11	Do not cohabitate potential non-target species which been described to potentially harbour the virus with koi.		Feasibility: Feasible for koi club members. Adoptability: The adoption is limited as many non-koi club members already cohabitate potential non-target species such as goldfish with koi. Changing this pattern of fish keeping is unlikely to achieve widespread adoption. Likelihood reduction: -0 (3)
Reduction of access of vermin (i.e. birds, rodent) and pets/farm animals to koi ponds/ tanks/ associated infrastructure. Risk rating: Medium (10)	12	Erect bird fences to eliminate birds/pets from entering ponds. Control rodents by good feed storage and bait stations.		Feasibility: Technically feasible Adoptability: Adoption likely limited by loss of aesthetic values and cost of erection and maintenance of equipment. Likelihood reduction: -1 (3)

Minimisation of contact with potentially contaminated equipment Risk rating: Medium (10)	13	Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should not be brought into the facility. Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry. Disinfect all equipment with chemicals at doses stated in Table 6 after each use.		Feasibility: Technically feasible Adoptability: Adoption may be less than 100% due to the cost of extra equipment and cost and safety of chemicals. Likelihood reduction: -1 (3)
Minimisation of risks associated with people/visitors/staff that may have been exposed to potentially contaminated water or diseased fish. Risk rating: Medium (10)	14	Disinfect hands and footwear upon entry (and exit) to a koi facility, and between separate areas of facility All visitors/contractors/researchers must be aware of the biosecurity required prior to being granted entry. Visitors must be accompanied by a staff member/owner at all times Visitor vehicles to be parked in dedicated parking area, preferably remote to fish stock and fish movement loading area Visitors are discouraged from touching walls tank/pond structures, water and/or fish		Feasibility: Not considered feasible to install footbaths at doors of hobbyist homes. Adoptability: Adoption limited by the practicality of the practice. People want to go outside to look at koi and feed koi not to disinfect their hands and footwear prior to looking at and feeding their koi. There is also a knowledge deficit regarding biosecurity among general members of the public. Education programs regarding biosecurity risks likely to be more effective in improving biosecurity awareness. However, access to this knowledge may be limited for non-koi club owners. Likelihood reduction: -1 (3)
Avoidance of feeding potentially contaminated food.	15	Feed sanitised food (i.e extruded pellets) only where possible. Do not feed materials		Feasibility: Not entirely feasible, as there are uses of raw diets in early life stages. Some low risk sources such as

Risk rating: Medium (10)	that has been potential contaminated with CyHV-3. Do not feed raw fish to koi.		vegetables, fruits, mealworms are used. Some higher risk feeds such as artemia, daphnia and bloodworms are fed. Adoptability: Unlikely to have industry wide adoption. Likelihood reduction: -0 (2)

B: Risks for pathogen (CyHV-3) spread within a koi facility

B: Risks for pathogen (CyHV-3) spread within a koi facility

Risk activity	#	Agreed approach	Suggestion made by FFVS	Stakeholder feasibility and adoption limitations
~~Management of fish movement within a facility.~~ Risk rating: Extreme (20)	~~1~~	~~None~~		Feasibility: Not feasible. Most hobbyist that have more than one pond have them within close proximity to one another. The aerosol and spill over (from splashing) is not prevented in many instances. Many koi owners have a single pond. Very few owners keep records of movements within and out of a facility. Adoptability: Not adoptable. Likelihood reduction: -0 (4)
~~Management of water between systems.~~ Risk rating: Extreme (20)	~~2~~	~~None~~		Feasibility: Not feasible for many koi hobbyists. Adoptability: Impractical for many hobbyists due to cost of equipment. Likelihood reduction: -0 (4)
~~Reduction of aerosol spread between ponds/tanks.~~ Risk rating:	~~3~~	~~None~~		Feasibility: Impractical as many ponds are located outdoors within close proximity. Aerosol spread cannot be controlled. Water may also be splashed by the koi into adjacent ponds. Adoptability: Not adoptable.

Extreme (20)				Likelihood reduction: -0 (4)
Minimisation of contact with potentially contaminated equipment Risk rating: High (15)	4	Access to quarantine zones is avoided where possible. Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry.		Feasibility: Most hobbyist indicated complete quarantine systems were not feasible to install. Adoptability: Adoption is likely limited by the cost of purchasing multiple sets of equipment, cost and human risk from handling potentially hazardous chemicals, cost and space required to hold fish for extended periods. Likelihood reduction: -1 (4)
Detection of latently infected fish Risk rating: Extreme (20)	5	None	Routinely sample skin mucus and gill mucus every six months/year and perform 2 PCR methods For ELISA tests, at least ten blood samples from separate individual koi are required. Apply stressors including transport, handling, temperature fluctuations and sampling stress 3-6 days prior to collection of samples. Likelihood reduction: -2 (4)	Feasibility: Technically feasible although limited laboratories with validated test available. Adoptability: Adoption limited by cost of diagnostic tests. Efficacy of the biosecurity measure limited by sensitivity of diagnostic tests. Likelihood reduction: -0 (4)
~~Vaccination of existing stock~~ Risk rating: Extreme (20)	~~6~~	~~None~~		Feasibility: Not feasible at this time due to no commercial availability of vaccines. It is not feasible for live attenuated vaccines to be used as it may be detrimental for the potential controlled release of CyHV-3. Adoptability: There could be low level adoption by the industry for high value show fish, but most likely, shows will cease and show fish will not be moved. The efficacy of vaccines is too poor for koi owners to justify use. IgY incorporated feed likely to be cost prohibitive. Cost of veterinary

				diagnostics and interpretation is prohibitive for most koi keepers. Reduction of stress is possible and avoided whenever possible for the koi. Likelihood reduction: -0 (4)
Prevention of spread of disease between systems/site and to another systems/site Risk rating: Extreme (20)	7	In the event of a material disease in a pond/system, all fish are culled. A decontamination process (i.e. hydrated lime (CaOH) application and drying of ponds in sunlight for a minimum of 7 days) must be undertaken prior to restocking. Decontamination must be undertaken in consultation with, or under instruction from, the relevant state government biosecurity agency to ensure compliance with biosecurity obligations or with a competent veterinarian. Fish movement is restricted for a minimum of one year (or until water temperatures fall below permissible ranges the following year). No new fish are permitted to be introduced into the site/fishery during the restriction period.		Feasibility: Technically feasible. Adoptability: The adoption is heavily dependent on the emotional and sentimental value of the koi. Culling of stock will likely result significant loss of genetic bloodlines which are difficult/ impossible to replace for the industry. For older keepers, culling is likely hobby ending. Some people may also not cull their stock as seen in the previous *Aeromonas spp* outbreaks where no fish were voluntarily culled. It appears unlikely that such measures could be rigorously implemented. Likelihood reduction: -1 (5)

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility				
Risk activity	#	Agreed approach	Suggestion made by FFVS	Stakeholder feasibility and adoption limitations
Management of effluent discharge Risk rating: High (15)	1	Ensure that all effluent discharge and their associated aerosol are separate from intake water and supply.		Feasibility: Some effluent waters are presently discharged into stormwater drainage. Adoptability: Stakeholder perception People are unlikely to change their method of discharging. Likelihood reduction: -1 (4)
Appropriate disposal of mortalities Risk rating: Low (5)	2	Fish mortalities are ~~recorded and~~ removed daily and disposed of by a method approved by the relevant authority, which ensures no risk of release of pathogens from the dead stock into waterways, or access for scavenger birds or animals (e.g. pigs, foxes, water rats) that could spread a disease.		Feasibility: Feasible, however, recording of mortalities unlikely to occur as owners are usually highly emotionally attached to their koi. Recording their death is not on their priorities when mortalities do occur. Adoptability: Easily adoptable. Likelihood reduction: -2 (3)
Management of people Risk rating: High (15)	3	None	Disinfect hands and footwear upon exiting the farm/site.	Feasibility: Not feasible to get guests to a home to rigorously complete biosecurity entry requirements. People may not wish to have disinfection protocols on entry and exit of a facility. Adoptability: Adoptability likely to be poor.

			Likelihood reduction: -0 (3)
Management of equipment Risk rating: Medium (10)	4	Dedicated equipment should be labelled and maintained for use exclusively on site. Dedicated equipment should not be removed from site and use for other purposes.	Feasibility: The feasibility may be affected by the cost of maintaining two sets of equipment for different purposes (i.e sharing of fishing equipment with koi keeping equipment). Adoptability: May be adoptable by the industry. Likelihood reduction: -1 (2)

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows				
Risk activity	#	Agreed approach	Suggestion made by FFVS	Stakeholder feasibility and adoption limitations
Additional disinfection protocols at koi shows/auctions Risk rating: Extreme (25)	1	None	For koi shows/auctions, additional disinfection with hydrogen peroxide based solution (i.e. Huwa-San©) at 60mg/L can be added into tanks, with care required in higher water temperatures (>20oC) if holding for longer than one hour Likelihood reduction: -4 (5)	Feasibility: Technically feasible. Research will be required to optimise the treatment in a koi show setting where fish may be held for upwards of 24 hours. Training and education will be required to teach people how to properly manage and handle the chemicals to appropriately calculate and use the chemicals. Adoptability: Adoption would be influenced by the safety of disinfectants such as Huwa-San©. The cost of the chemicals is also likely to affect whether the chemicals are used or not. Likelihood reduction: -0 (5)
Avoidance of certain show formats Risk rating: High (15)	2	Where possible, do not cohabitate koi or share water with koi from different systems/facilities/premises/ponds/tanks together with other koi. Where possible, adopt the English style layout for all koi shows to prevent risk of horizontal transmission of CyHV-3 through contact with infected fish.	Use of disinfectants such as Huwa-San© at 60mg/L to reduce risk of disease movement through splashing of water or by misplacement of koi into wrong tanks/bins by members of public. Likelihood reduction: -4 (5)	Feasibility: Koi shows all follow the English style of shows. It may be difficult to prevent accidental cohabitation by fish that jump into adjacent tanks/bins or from fish that are mistakenly placed into the wrong tanks/bins by members of the public when they jump out of the water. Water can be accidentally spread as fish may splash water and spread water to adjacent, nearby tanks/bins. Adoptability: Adoption is likely. Likelihood reduction: -3 (5)
Disinfection and disposal of transport	3	None	Use of disinfectants such as Huwa-San© at 60mg/L to reduce risk of transmission	Feasibility: For most koi keepers, bags are likely to be floated and fish are released with their associated waters back into the

water returning from auctions/shows Risk rating: Extreme (25)		of disease through potentially contaminated water from koi shows	main ponds. It is too physically difficult for older members to transport koi fish multiple times. Some people are likely to discharge water on the ground/driveway. Adoptability: Unlikely to be adopted. Likelihood reduction: -0 (5)
Screening of koi prior to shows/auctions Risk rating: Extreme (20)	4 Koi fish that visibly appear sick are not permitted to attend shows/auctions		Feasibility: It is feasible for koi to be visually inspected prior to attending a show/ auction. This is currently performed by club members. Adoptability: Adoption is easy as it is already performed. Limitations of this process if that subclinical fish are more likely to attend auctions/shows. Once they are at auctions/shows they pose a significant risk to other koi. Likelihood reduction: -1 (5)
~~Risk rating:~~ ~~Extreme (25)~~	~~Current veterinary health certificates and diagnostic results must be obtained prior to placing koi into shows/auctions~~		Feasibility: The cost of veterinary diagnostics and certifications is too costly for shows and auctions. The cost of the fish is unlikely to be over the price of the diagnostics and veterinary examinations to yield any profit at auctions. Auction sale profits make up a significant portion of funding for koi shows. Without profit at koi auctions, shows will not occur, and the hobby is unlikely to continue. Adoptability: Not adoptable. Likelihood reduction: -0 (5)

Limiting of koi permitted to shows/auctions Risk rating: Extreme (25)	5	None	Koi from facilities with a history of CyHV-3 exposure are not permitted to attend shows/auctions unless all stock are culled, and facilities are disinfected and restocked with certified CyHV-3 free stock. Likelihood reduction: -3 (5)	Feasibility: Use of vaccinated fish is not feasible at this time due to no commercial availability of vaccines. It is not feasible for live attenuated vaccines to be used as it may be detrimental for the potential controlled release of CyHV-3. Adoptability: Adoption of vaccination is unlikely to occur. Shows/auctions will likely cease if CyHV-3 was released in Australia as the risk is deemed too high by many koi keepers. Loss of fish from disease or from culling will be detrimental to Australia's limited genetic bloodlines especially as they will be difficult/ impossible to replace. Likelihood reduction: -0 (5)
Reduction of transmission risks associated with equipment usage Risk rating: Low (5)	6	Sharing of equipment such as nets is prohibited. Disinfect all equipment between use.		Feasibility: It is feasible for each person to have their own equipment that is not shared. Adoptability: This option is likely easily adoptable. Likelihood reduction: -3 (4)
Reduction of cross contamination by people Risk rating: Medium (10)	7	Contact with water, equipment or fish during shows/auctions is discouraged (signs, physical barriers).	Additional compulsory disinfection procedures (i.e alcohol hand spray on entry, similar to petting zoos) Likelihood reduction: -3 (4)	Feasibility: Technically feasible. Adoptability: Children and less biosecure aware members of the public may not follow signs and instructions. Likelihood reduction: -2 (4)

Appendix 1: Table 6 – Disinfection dosages against CyHV-3

Disinfectant	Treatment time	Temperature (15ºC)	Temperature (25ºC)	Temperature (unspecified)
Iodophor (mg/L)	30 s	200	200	N/A
	20 min	200	200	N/A
Sodium hypochlorite solution (mg/L)	30 s	>400	>400	N/A
	20 min	200	250	N/A
Benzalkonium chloride solution (mg/L)	30 s	60	30	N/A
	20 min	60	30	N/A
Ethyl alcohol (%)	30 s	40	30	N/A
	20 min	30	25	N/A
Free Chlorine (mg/L)	30 min	N/A	N/A	3

Table 28 Chemicals used for disinfection of CyHV-3 adapted from Kasai et al. 2005.

Appendix 7 – Phase 3 Meeting minutes

Future Fisheries
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Meeting minutes with stakeholders of the koi industry for hobbyist.

Dr Chun-han Lin BVSc(Hons)

Dr Matt Landos BVSc(HonsI)MANZCVS

3 July 2019

Meeting minutes for meeting on 25/6/19:

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

A1:

Feasible and adoptable for industry to use town-water or bore-water as water source for koi keepers. Questions were raised regarding the potential of chlorination failure at chlorination plants

A2:

Not feasible for industry to use UV light due to cost and inability for fine filtration of water. The stakeholders are wishing to understand the impact of salinity on CyHV-3 infectivity and viability. Further research will be required to further understand this aspect.

A3:

Not feasible or adoptable

A4:

Testing and recording of temperature, pH, TAN, nitrite, nitrate, algal bloom (visually) **weekly** is feasible for most members of the koi club. Daily monitoring is not feasible. Education is likely required for wider adoption (i.e water quality information guidelines published by the Government). There may be some resistance from some koi club members to perform routine water quality testing due to on going costs. Financial support for test kits may assist these members in adopting regular testing.

A5:

It is feasible and adoptable for koi keepers to use town-water or bore-water to prevent potentially infected mollusc, plankton, crustacea from entering koi ponds/tanks.

A6:

It is feasible and adoptable for koi keepers to use town-water or bore-water to prevent wild carp entry into ponds/tanks.

It is not adoptable by the industry to erect netting around their ponds at all times as it is aesthetically unappealing. Some koi keepers design their backyards so that their ponds are the main feature. Members of the koi club can erect netting when predatory birds are more prevalent. Further research into the infectivity and viability of CyHV-3 from regurgitate/digested/faecal matter of birds that ingest infected carp/koi will assist in adoption of netting.

A7:

The only feasible and adoptable option for koi keepers is to source fish from disease free facilities with known histories. Vaccination and certifications are not viable for the industry. The current level of protection achieved by vaccination is poor for the industry. The current diagnostic procedures are not sensitive enough for subclinically infected, latent carriers of the disease for the industry to adopt the testing for certification. It was raised that certifications have been employed overseas with no success.

A8:

Most koi keepers do not have access to and/or ability to quarantine koi. Quarantine is not a feasible option. Many koi keepers do not have the knowledge to recognise abnormalities even if there is access to quarantine facilities. Diseases such as KHVD may potentially be missed. Training and education may assist keepers that do have access to quarantine facilities to recognise diseases. For equipment, it is feasible and adoptable for koi club members to use dedicated equipment and disinfect when there are no other options with disinfectants such as sodium hypochlorite and Virkon S. Training, education on handling of chemicals and public awareness of risks associated with using potentially infected equipment will facilitate in the adoptability of this option.

A9:

The sensitivity of the current diagnostic methods is too low for the koi industry to adopt testing of sick/ freshly dead fish and for surveillance. The cost of diagnostic tests may impact the feasibility of the option. Further research into a more sensitive test that can be performed non-lethally and collected easily will assist in adoption of diagnostic testing.

A10:

The only feasible and adoptable option for the industry currently is to ensure that the health status of all new fish is adequate before stocking with existing stock. There is unlikely to be any adoption by the industry due to the sensitivity of current diagnostic methods. The cost of diagnostic tests may impact the feasibility of the option. Further research into a more sensitive test that can be performed non-lethally and collected easily will assist in adoption of diagnostic testing.

A11:

It is feasible and adoptable for koi club members to not cohabitate potential non-target species (NTS) with koi. However, this may not be possible for non-koi club members. Public education may assist in adoption by general members of the public, but it is unlikely to be 100% efficacious.

A12:

It is not adoptable by the industry to erect netting around their ponds at all times as it is aesthetically unappealing. Some koi keepers design their backyards so that their ponds are the main feature. Members of the koi club can erect netting when predatory birds are more prevalent. Further research into the infectivity and viability of CyHV-3 from regurgitate/digested/faecal matter of birds that ingest infected carp/koi will assist in adoption of netting.

A13:

For equipment, it is feasible and adoptable for koi club members to use dedicated equipment and disinfect when there are no other options with disinfectants such as sodium hypochlorite and Virkon S. Training, education on handling of chemicals and public awareness of risks associated with using potentially infected equipment will facilitate in the adoptability of this option.

A14:

There is unlikely to be any adoption by koi keepers to disinfect hands prior to entry and exit of a koi facility each time. It is potentially feasible for koi keepers to enforce disinfection of hands for people that are wishing to touch fish or equipment.

Visitors already park away from ponds and are accompanied by owners. These options are feasible and adoptable.

It is not feasible or adoptable for koi keepers to enforce visitors to change into freshly laundered clothes or site provided clothing.

Biosecurity declaration, signage, and visitor logbooks are unlikely to be kept and are not adoptable or feasible for the industry. It is unlikely that a biosecurity induction will be given to any visitors. This is considered not feasible or adoptable for the industry.

A15:

It is not feasible for the industry to avoid unsanitised food (i.e artemia, daphenia, bloodworms) during early life stages. Further research into the whether these organisms are infective carriers/ vectors (both biological and mechanical vectors) may assist koi keepers in assessing the risk of feeding these feed sources. For later life stages, extruded pellets are fed and it is feasible and adoptable for the industry to feed sanitised food.

B: Risks for pathogen (CyHV-3) spread within a koi facility

B1:

Not feasible or adoptable.

B2:

Not feasible or adoptable.

B3:

Not feasible or adoptable.

B4:

Many koi keepers do not have access to or are unable to hold fish in quarantine. This option is considered not feasible as a result. For equipment, there is a knowledge gap for koi keepers in handling and using chemicals safely, and training and education is likely required for adoption.

B5:

The sensitivity of the current diagnostic methods is too low for the koi industry to adopt testing for surveillance. The cost of diagnostic tests may impact the feasibility of the option. Further research into a more sensitive test that can be performed non-lethally and collected easily will assist in adoption of diagnostic testing. With the current test sensitivities, koi keepers are likely to perform visual infections on their fish without any diagnostic or veterinary input. Social media is likely to be used. As mentioned previously, there is a knowledge gap for koi keepers in recognising disease, potentially, diseases such as KHVD may be missed.

B6:

It is not feasible for owners to inject and handle fish multiple times. There is likely to be more adoption if the vaccine was given as an immersion bath or orally, however, the efficacy will likely have to be high (~99%) for at least 12 months before there is any adoption by the industry.

B7:

Difficult to enforce culling for everyone especially if they are of high sentimental value. The koi clubs are wanting a compulsory reporting system and culling to be enforced by the government for CyHV-3 such that the koi clubs are able to enforce stricter biosecurity measures around affected populations. The koi club is likely to provide aid to any members whose entire collection is culled as a result of KHVD. There is likely to be a 12 month transport and trading restriction placed on the affected members (i.e not able to show any fish, not able to sell or trade any fish). For members whose collection is not completely culled, that member is likely to be excluded from all activities hosted by the club.

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility

C1:

It is feasible and adoptable for koi club members to ensure that their effluent discharge and aerosols are separate from their intake water source. For non-koi club members, it may be difficult for widespread adoption. Education programs may assist in wider adoption but is unlikely to achieve 100% efficacy.

C2:

Appropriate disposal of mortalities is feasible for koi club members. It is not feasible for koi club members to record mortalities. For non-koi club members, it may be difficult for widespread adoption. Education programs may assist in wider adoption but is unlikely to achieve 100% efficacy.

C3:

It is not feasible or adoptable for disinfection to occur on footwear and hands upon exit of a facility.

C4:

There may not be industry wide adoption of labelling as some people only have one set of equipment. Equipment are kept exclusively for koi use for koi club members. For non-koi club members, it may be difficult to get adoption of disinfection or for them to maintain equipment for dedicated use. Education programs may assist in wider adoption, but it is unlikely to achieve 100% efficacy.

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows

D1:

The option to add disinfectants such as Huwa-San © is feasible for the industry. Currently there is a knowledge deficit restricting the adoptability of this option. Research into the long and short term effects of disinfectants on koi will likely be required and deemed safe before there is any adoption by the industry. The current study only extends for an 8 hour duration which is inadequate for many koi shows as fish may be held on the site for up to 24 hours. Training will be required for koi keepers to understand how the chemical is handled and used safely.

D2:

It is feasible and adoptable for the industry to adopt English style shows and it is currently performed.

It may be difficult to prevent members of the public from accidentally placing fish in wrong tanks/bins or fish from splashing water to adjacent tanks/bins. It may be feasible to add disinfectants such as Huwa-San © into tanks/bins to reduce those risks. Currently there is a knowledge deficit restricting the adoptability of this option. Research into the long and short term effects of disinfectants on koi will likely be required and deemed safe before there is any adoption by the industry. The current study only extends for an 8 hour duration which is inadequate for many koi shows as fish may be held on the site for up to 24 hours. Training will be required for koi keepers to understand how the chemical is handled and used safely. It is adoptable for the industry to install splash guards or obtain deeper tanks/bins to prevent/reduce risk of splashing or fish from jumping out, however, the feasibility of this option is restricted due to cost.

D3:

It is unlikely to be feasible for the whole industry to dispose of all waters from shows. It may be feasible for the industry to use disinfectants such as Huwa-San ©. Currently there is a knowledge deficit restricting the adoptability of this option. Research into the long and short term effects of disinfectants on koi will likely be required and deemed safe before there is any adoption by the industry. Training will be required for koi keepers to understand how the chemical is handled and used safely.

D4:

It is feasible and adoptable for the industry to not permit fish that are visibly sick from attending shows/auctions. There may be limitations for koi club members in identifying diseases and a veterinarian may be required, however the cost of veterinary inspection and surveillance is likely to restrict the feasibility of the option at shows and auctions.

It is not feasible for the industry to perform diagnostic tests as it will not reflect the current status of the fish and are unlikely to be sensitive for latently infected subclinical carriers of CyHV-3. The use of rapid detection kits such as lateral flow devices to detect CyHV-3 may be adoptable for the industry if it was sensitive for clinically affected and subclinically infected carriers of CyHV-3.

D5:

The koi clubs are seeking for compulsory reporting and culling of koi populations affected by CyHV-3. There is likely to be a one year movement and trade restriction applied to affected members by the koi club. For koi club members that have not culled their entire populations, a permanent movement restriction and trade restriction is likely to be enforced by the koi club for any koi club hosted activities.

D6:

Currently, nets are labelled at shows and no sharing of equipment takes place. It is feasible and adoptable for the industry to implement disinfection protocols for equipment. Education and training regarding safe handling of chemicals is likely required for the option to be adoptable and feasible.

D7:

It is feasible and adoptable for the industry to discourage people from contacting water, fish and equipment during shows and auctions. However, it is unlikely to achieve 100% efficacy.

Other comments:

- Culling of stock may be hobby ending for some members.
- Addition risk was identified for shows and auctions with water transport commercial tankers. It is feasible for the industry to enforce disinfection of the tanker; however, the adoptability may be limited if adoption is not performed by the contractor. Addition disinfection with Huwa-San © may be feasible for the industry. Currently there is a knowledge deficit restricting the adoptability of this option. Research into the long and short term effects of disinfectants on koi will likely be required and deemed safe before there is any adoption by the industry. Training will be required for koi keepers to understand how the chemical is handled and used safely.
- Some koi farms do attend auctions and shows.
- Only one koi farm has responded and has been available to comment on the feasibility and adoptability of options.

Appendix 8 – Phase 4 Estimate costing

Estimate cost to implement feasible biosecurity and control strategies against Cyprinid Herpesvirus 3 (CyHV-3) identified by stakeholders for the hobbyist and commercial farming industry in Australia in relation to the potential release of the virus as a biocontrol agent.

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22 July 2019

Contents

1.0 Introduction

Cyprinid Herpesvirus 3 (CyHV-3) also known as Koi Herpesvirus (KHV) is an OIE notifiable disease in koi (*Cyprinus carpio koi*) and European carp (*Cyprinus carpio*). CyHV-3 is the pathogen responsible for causing Koi Herpesvirus Disease (KHVD). The term disease describes the damaged tissues which result from viral replication. In subclinical infections with CyHV-3, no tissue damage may be evident, hence the fish is not technically diseased. It is however an infected carrier of the virus which could potentially spread to other susceptible fish. In recent years, CyHV-3 has been explored as a possible biocontrol measure for European carp in Australia. This virus is presently exotic to Australia. It has been reported internationally in 33 countries including Canada, Indonesia, Japan, Taiwan, Thailand, Poland, Singapore, United Kingdom, and the United States of America.

The potential release of CyHV-3 into Australian waters would introduce new risks to Australia's koi industry. A literature review and biosecurity options list were compiled to assess and explore potential risk mitigation strategies against CyHV-3. A stakeholder meeting in Sydney was held to explore each biosecurity options identified from the literature review.

It was identified during the meeting by stakeholders that the risks and mitigation strategies against CyHV-3 differs vastly between koi hobbyists and koi farmers. Current Australian koi farmers were contacted regarding the project to seek their participation via phone discussions. Only one of the identified four commercial koi farmer offered responses. Further subsequent attempts to contact other koi farmers were unsuccessful. Therefore the industry views in this report, with respect to koi farm level biosecurity, may not reflect the adaptability of biosecurity options for the entire industry in Australia.

A teleconference with the koi industry steering committee was held on the 25th June 2019 and the feasibility and adoptability of each option was discussed. Meeting minutes were distributed to relevant stakeholders following the meeting.

Approximate cost estimates to implement feasible strategies identified by stakeholders were obtained through consultation with equipment suppliers, chemical suppliers, designers, and with relevant government bodies and laboratories.

Entity to whom costs would directly fall (assuming nil Government subsidy)

Industry highlighted that many of the biosecurity measures identified and costed below, would be out of reach financially for many of them. Whilst the table notionally indicates costs that would fall to industry, the actual cost would vary with the capacity of industry to take up the measures, depending on whether Government assistance for industry was involved. The costs have been identified from commercial suppliers of products and based on the assumptions outlined above.

Costs have been separated into four categories and colour coded as outlined below:

Industry borne cost- one off (anticipated to have life-span > 5 years)
Industry borne cost – recurring
Government borne cost- one-off
Government borne cost - recurring

2.0 Cost estimation of feasible biosecurity options for commercial koi farms

Assumptions:

Farm size: 10 hectares. 10 x 9ML ponds

Annual volume of water use:

Peak farm intake flow rate: 208,333L/hr

Frequency of new domestic fish intake (presently koi are not a permitted import to Australia): No new entry of fish into founder population

Labour cost: $40/hr

All costs are exclusive of GST

KHV Surveillance metrics: 150 fish every 6 months, for 2 years duration

PCR test cost per sample (one fish and single target tissue): First sample $395 +197.50= $592.50, further samples $52 and $26= $78, If positive, then further confirmatory testing prices first sample (one fish and single target tissue) $360, then $46.00

Histology test cost: $150.00/fish

Veterinary field cost: $160/hr

Sentinel koi (which are fish that are known to be CyHV-3 free, that are purchased solely for the purpose of cohabitating with the farmers population of koi which have an unknown disease status. Post-cohabitation the sentinels are terminally sampling for laboratory testing. This avoids the need to terminally sample the valuable farm stock, whilst providing an indication of their likely disease status): $5/fish

Fish vaccination needles: $40/ 12 pack

** denotes where the cost is a repeat of an expense already detailed with one of the previous risk activity feasible options.

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility				
Risk activity	#	Feasible option	Concerns raised by stakeholders	Estimated Cost
Water source (and organisms entrained within) to farm/pond/tank.	1	Bore-water is used wherever possible. Where the use of bore-water is not feasible, lake/river/small dam waters are used with disinfection/ decontamination protocols applied.	The use of lake/river/small dam waters is currently used, however there is a severe limitation on cost of implementing a chemical decontamination protocol. Businesses perceive a lack of any return on investment.	**Cost of chemical decontamination of water (90ML):** Sodium hypochlorite 12.5% (to achieve 3mg/L): $13,000 Cost of Chlorine photometer: $2,100 **Annual test kit reagent cost**: $400
Disinfection/deactivation of CyHV-3 in incoming water.	2	None without the aid of subsidy.	The cost of disinfection units and filtration units are prohibitive for the industry. The cost of chemical disinfection is prohibitive for the industry. To implement a disinfection protocol for incoming waters, significant investment must be made by the business which is unlikely to see any return on investment.	**Cost of fine filtration and UV disinfection (4mJ/cm²) of water:** **26 micron drum filter:** $30,000 **Drum screen annual service:** $2000 **UV unit:** $20,000 **UV globe annual replacement**: $3000
Management of water quality	4	Testing daily: Dissolved oxygen (DO), Temperature, pH, Salinity, Total Ammonia Nitrogen (TAN), algal blooms to maintain good water quality. Maintain clean ponds.	Water quality testing may not be routinely performed. Time, staff availability, cost of equipment are limiting factors	**Cost of water quality test meters and test kits:** $4,500 **Annual probe replacement cost**: $400 **Cost of compound microscope with camera:** $2,800 **Cost of labour: 1hr/day 365 days per year:** $14,600

Prevention of potentially infected planktons, molluscs and crustaceans entry into ponds/tanks.	5	Bore-water is used wherever possible. Where the use of bore-water is not feasible, lake/river/small dam waters are used with disinfection/ decontamination protocols applied.	Fine filtration (down to 5 microns) likely to restrict flow on the farm. Cost of filtration, equipment and pumping likely to be a limiting factor for filtration. Bore-water quality may vary dramatically, and treatment of the water may be too expensive for businesses. Where the use of bore-water is feasible, would be the preferred option. The use of chemicals is cost prohibitive.	**Cost to analyse bore-water quality:** $198 **Cost of chemical disinfectants:** Sodium hypochlorite 12.5% (to achieve 3mg/L): $13,000** Cost of Chlorine photometer and reagents: $2,100 **Annual test kit reagent cost**: $400
Prevention of wild carp entry into ponds/tanks.	6	Ensure that adequate filtration is in place to ensure that no carp/eggs can enter the system through intake water.	Fine filtration likely to restrict flow on the farm. Cost of filtration and pumping likely to be a limiting factor for this option.	**Cost of filtration to exclude carp entry:** **26 micron drum filter:** $30,000** **Drum screen annual service:** $2000
		Ensure adequate fencing/netting are in place to prevent bird access to ponds/tanks.	The use of nets is limited to when fish are small and are at higher risk of predation. The cost of netting is a limiting factor for farms. Netting also require constant maintenance for the farmers. Often wildlife can become entangled in netting especially after storms which requires time and money to release them from the nettings.	**Cost of bird nets per 3-5 years:** $750,000 **Cost of fencing:** $30,000 **Cost of labour to maintain netting and fencing (1hr/week):** $4,160
Introduction of new koi onto premise	7	None	Cost of obtaining SPF status is unlikely to yield any economic benefits for farms in Australia. No SPF stock available in Australia.	**Cost to achieve Specific Pathogen Free (SPF) status (2 years of molecular and histology testing inclusive with 150 randomly selected samples from entire farm at each sample point every 6 months):**

			Molecular surveillance: $48,858 (if no positives) - $77,714 (if positives are detected in confirmatory testing) Histology: $90,000 **Maintenance of SPF annual diagnostic cost:** Biosecurity audit (per farm): $5000.00
	None	Cost of diagnostic tests likely to exceed the value of fish and make the business unprofitable. Sourcing fish from areas without a history of CyHV-3 poses significant risk as it relies on trust and people may not be aware of their fish's disease status. The efficacy of the vaccines is unlikely to yield any benefits to the business. The stakeholder has not introduced any new koi to the collection.	**Cost of diagnostic tests:** Veterinarian to collect samples: $320 Travel: $500 PCR (single imported fish and thirty sentinels): $2,932.50 if all negative, $4,672.50 if suspect positives require confirmation. Sentinel koi: $150 Histology (30 sentinel fish): $4,500 Veterinary pathology interpretation: $160 **Research cost for development of an optimised KHV vaccine and concurrently prepare data package for an APVMA MUP application:** $750,000 **See report on additional koi industry research needs.** **Cost to vaccinate fish:** Labour (for 20,000 fish): $5,000

				Vaccine table: $4,300
				Vaccine guns (6): $1,200
				Vaccine gun needles: $80
				Anaesthetic (AQUI-S): $500
				Off-label veterinary prescription: $120.00
				Oxygen (Size E 4.1m³): $305
				Ceramic Airstones (3): $720
				Water quality monitoring meters: $4,500**
				Annual probe replacement cost: $400**
				Vaccine: $20,000-$60,000 (for 20,000 fish per year)
Duration of quarantine period	8a	None	The stakeholder does not have a dedicated quarantine facility and have not introduced any new broodstock or koi to the farm. The cost of setting up a dedicated quarantine facility is considered financially unjustifiable.	**Cost of setting up a dedicated quarantine facility:** Concrete floor shed: $100,000 Two 5,000L tanks: $4,980 Oxygen generator: $1,900 Ozone (8g/hr): $2,700 UV (40W 4mJ/cm²): $500 Annual replacement lamp: $300 Heater/Chiller for 10,000L system: $9,000 Microscope with camera: $2,800** Hand Nets: $400

				Bins/Buckets: $56 Chlorine Photometer: $2,100** Annual test kit reagent cost: $400** Water quality meters: $4,500** Annual probe replacement cost: $400** Footbath: $300 Hand spray: $1.50 Disinfection tub: $50 Disinfection chemicals: $230 Dissection equipment: $50 Formalin jars (50): $80 80% Ethanol (1 litre): $60 Rubber boots (5 pairs): $200 **Cost of maintaining fish in quarantine for 3 weeks:** Labour: $1,680
Cohabitation with sentinel koi	8b	None	Cost of purchasing and maintaining 10-30 sentinel koi is uneconomic for the business. There are currently no sentinel koi available in Australia.	**Cost of locally sourced CyHV-3 free sentinel koi fish (30 sentinel koi):** $150
Quarantine temperature ranges	8c	None	The cost of equipment and electricity to heat and cool water and space required to	**Cost of heater and chiller for 10,000L system:** $9,000**

			maintain permissive temperatures for pathogen transmission and for KHVD is too costly.	
Monitoring of quarantine koi	8d	Observe quarantine fish daily for any abnormal observations.	The time required to document findings is too time consuming for the stakeholder. The stakeholder has never introduced new koi to founder population.	**Cost to monitor and record findings (2 weeks full time):** Labour: $4,480
Selection of quarantine location	8e	Monitor all new and returning fish in a dedicated quarantine system for signs of disease.	The cost of setting up a dedicated quarantine facility is financially unjustifiable.	**Cost of setting up a dedicated quarantine facility:** Concrete floor shed: $100,000 Two 5,000L tanks: $4,980 Oxygen generator: $1,900 Ozone (8g/hr): $2,700 UV (40W 4mJ/cm^2): $500 Annual replacement lamp: $300 Heater/Chiller for 10,000L system: $9,000 Microscope with camera: $2,800** Hand Nets: $400 Bins/Buckets: $56 Chlorine Photometer: $2,100** Annual test kit reagent cost: $400** Water quality meters: $4,500**

				Annual probe replacement cost: $400**
				Footbath: $300
				Hand spray: $1.50
				Disinfection tub: $50
				Disinfection chemicals: $230
				Dissection equipment: $50
				Formalin jars (50): $80
				80% Ethanol (1 litre): $60
				Rubber boots (5 pairs): $200
Additional quarantine barriers (dedicated equipment and disinfection of equipment)	8f	All quarantine equipment remains in quarantine area.	Cost of purchasing dedicated quarantine equipment.	**Cost of additional quarantine equipment:**
				Microscope with camera: $2,800**
				Nets: $400**
				Buckets: $56**
				Chlorine Photometer: $2,100**
				Annual test kit reagent cost: $400**
				Water quality meters: $4,500**
				Annual probe replacement cost: $400**
				Dissection equipment: $50**
				Rubber Boots (5 pairs): $200**

	8g	Where no alternative exists, and the use of non-dedicated equipment is required, non-dedicated equipment should be disinfected with chemicals described in Table 6 prior to use.	Storage space required to store chemicals if large quantities are required.	**Cost of implementing hand and footwear disinfection protocol:** **Chemical:** Ethanol (70%) (5L): $180 Virkon S (1kg): $100 Sodium hypochlorite 12.5% (20L): $100 **Equipment:** Hand spray bottle: $1.50** Footbath: $300** Disinfection tub: $50** **Cost to develop disinfection protocols for koi farms:** Development of protocols (7 days veterinary consulting): $10,500 Farm visits for implementation (4 farms, 1 day per farm): $9,000 Travel: $3,000
Deliberate stressors during quarantine	8h	None	The cost of setting up a quarantine facility and for diagnostic tests is uneconomic for the stakeholder. The space required to hold fish is limited on the stakeholder's farm.	**Cost of diagnostic tests:** Veterinarian to collect samples: $320** Travel: $500**

PCR (30 sentinel koi): $2,932.50 (if all test negative)-$4,672.50 (if test positive fish need to have confirmatory testing undertaken)**

Sentinel koi: $150**

Histology (30 sentinel koi): $4,500**

Veterinary interpretation: $150**

Cost of setting up a dedicated quarantine facility:

Concrete floor shed: $100,000

Two 5,000L tanks: $4,980

Oxygen generator: $1,900

Ozone (8g/hr): $2,700

UV (40W 4mJ/cm^2): $500

Annual replacement lamp: $300

Heater/Chiller for 10,000L system: $9,000

Microscope with camera: $2,800**

Hand Nets: $400

Bins/Buckets: $56

Chlorine Photometer: $2,100**

Annual test kit reagent cost: $400**

Water quality meters: $4,500**

				Annual probe replacement cost: $400** Footbath: $300 Hand spray: $1.50 Disinfection tub: $50 Disinfection chemicals: $230 Dissection equipment: $50 Formalin jars (50): $80 80% Ethanol (1 litre): $60 Rubber boots (5 pairs): $200 **Cost to develop sampling protocols for koi farms:** Development of protocols (7 days veterinary consulting): $10,500 Farm visits for implementation (4 farms, 1 day per farm): $9,000 Travel: $3,000
Disease investigation/surveillance of sick/ freshly dead quarantined fish	9	None	Cost of diagnostic tests is unviable for the business. Diagnostic tests do not provide 100% exclusion. Contact with veterinarians is infrequent and only sought after significant disease has occurred and the stakeholder is unable to identify a plausible causation factor.	**Cost of diagnostic tests:** Veterinarian to collect samples: $320 Travel: $500 PCR (three sick fish) $748 if all negative,

				$1200 if suspect positives require confirmation. Histology (3 sick fish): $450 Veterinary pathology interpretation: $160 **Cost to develop sampling protocols for koi farms:** Development of protocols (7 days veterinary consulting): $10,500** Farm visits for implementation (4 farms, 1 day per farm): $9,000** Travel: $3,000**
Health status of new fish	10	Ensure that the health status of all new fish is adequate before stocking with existing stock.	Concerns were raised regarding the cost of diagnostic tests if stocking was required. Fish are likely to be stocked if they look of sufficient health status if the cost of diagnostic tests are too high. Limited diagnostic laboratories available in Australia for CyHV-3. Diagnostic tests do not provide 100% exclusion.	**Cost of diagnostic tests:** Veterinarian to collect samples: $320** Travel: $500** PCR (single imported fish and thirty sentinels): $2,932.50 if all negative, $4,672.50 if suspect positives require confirmation. Sentinel koi: $150** Histology (30 sentinel fish): $4,500** Veterinary pathology interpretation: $160** **Cost of physical examination of fish**:

				Veterinary exam: $320 Travel: $500 Veterinary report: $160 **Cost to develop sampling protocols for koi farms:** Development of sampling protocols (7 days veterinary consulting): $10,500** Farm visits for implementation (4 farms, 1 day per farm): $9,000** Travel: $3,000**
Reduction of risk associated with cohabitation/stocking of potential non-target species	11	Do not cohabitate potential non-target species which been described to potentially harbour the virus with koi.	The stakeholder stocks silver perch and goldfish on site. Cohabitation is not performed in the grow out ponds, however, it is common for goldfish and koi to be cohabitated together in display tanks. The airspace between ponds are shared. No disinfection protocols are in place for recirculated waters that move between tanks which contain different species. The option to remove non-koi species from the farm is not feasible due to the economic impact it would have. The cost to make the areas biosecure is not cost-effective.	**No additional cost to stakeholder.**
Reduction of access of vermin (i.e. birds, rodent) and pets/farm	12	Erect bird fences to eliminate birds/pets from entering ponds. Control rodents by good feed storage and bait stations.	Feasibility: It is difficult to exclude all animals from entering the ponds even with netting and fencing. Farm animals such as dogs and cattle require substantial fences,	**Cost of bird nets 10ha per 3-5 years:** $750,000 **

animals to koi ponds/ tanks/ associated infrastructure.			not presently in place, to reliably exclude from pond access. Many farms have bird netting in place. However, it is impractical for keeping small birds such as kingfishers out of ponds. Netting may not be used at all times as wildlife often become entangled in netting especially after storms which requires time to release them from the netting. Netting and fencing will not keep turtles and frogs out of ponds. Adoptability: Time required to maintain netting and fencing and the cost to replace netting and fencing are uneconomic for the business	**Cost of fencing:** $30,000 ** **Cost of labour to maintain netting and fencing (1hr/week):** $4,160**
Minimisation of contact with potentially contaminated equipment	13	Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should not be brought into the facility. Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry.	Feasibility: It is possible for dedicated equipment to be used on site. Stakeholder shares equipment throughout the facility. Used equipment are sun dried after each use. If the equipment is dirty, then they are cleaned with detergent prior to being sun dried. All equipment are disinfected with hypochlorite at the end of the season. Adoptability: Stakeholder raised concerns regarding the cost of chemicals and dedicated equipment. Concerns were raised regarding buyers and contractors. It is difficult to enforce buyers and contractors to disinfect their equipment and disinfection is unlikely to occur. It is believed that enforcing these rules will only result in loss of sales.	**Cost of implementing hand and footwear disinfection protocol:** **Chemical:** Ethanol (70%) (5L): $180** Virkon S (1kg): $100** Sodium hypochlorite 12.5% (20L): $100** **Equipment:** Hand spray bottle: $1.50** Footbath: $300** Disinfection tub: $50**

				Cost to develop disinfection protocols for koi farms: Development of customised disinfection protocols for koi farms (7 days veterinary consulting): $10,500 Field implementation of disinfection protocols (4 farms @ 1 day per farm): $9,000 Travel: $3,000 **Cost of purchasing dedicated equipment:** Water quality meters:$4,500** Annual probe replacement cost: $400** Buckets: $56** Hand Nets: $400** Rubber Boots (5 pairs): $200**
Minimisation of risks associated with people/visitors/staff that may have been exposed to potentially contaminated water or diseased fish.	14	Disinfect hands and footwear upon entry (and exit) to a koi facility, and between separate areas of facility Staff/owners must wear freshly laundered clothes each day prior to entry into a koi facility. All visitors/contractors/researchers must be aware of the biosecurity required prior to being granted entry.	It is difficult to change human behaviour. The stakeholder believes that implementing all of the measures will discourage his buyers from purchasing fish from him and will go elsewhere or seek the black-market for fish.	**Cost of implementing hand and footwear disinfection protocol:** **Chemical:** Ethanol (70%) (5L): $180** Virkon S (1kg): $100** Sodium hypochlorite 12.5% (20L): $100** **Equipment:** Hand spray bottle: $1.50** Footbath: $300**

				Disinfection tub: $50**
		Visitors must be accompanied by a staff member/owner at all times		
		Visitor vehicles to be parked in dedicated parking area, preferably remote to fish stock and fish movement loading area where possible.		**Cost to develop disinfection protocols for koi farms:** Development of customised disinfection protocols for koi farms (7 days veterinary consulting): $10,500** Field implementation of disinfection protocols (4 farms @ 1 day per farm): $9,000** Travel: $3,000**
				Cost to maintain visitor logbook: $25
Avoidance of feeding potentially contaminated food.	15	Feed sanitised food (i.e extruded pellets) only. Do not feed materials that has been potential contaminated with CyHV-3.	Easily adoptable.	**No additional cost to stakeholder.**
		Do not feed raw fish to koi.		

B: Risks for pathogen (CyHV-3) spread within a koi facility

B: Risks for pathogen (CyHV-3) spread within a koi facility				
Risk activity	#	Feasible option	Concerns raised by stakeholders	Estimated cost
Management of fish movement within a facility.	1	None	The stakeholder noted that record keeping of all fish movement on site, between areas is time consuming and believes that there is very little financial reward.	**Annual cost to record fish movement (1hr/week):** $2,080
Management of water between systems.	2	Where water is recirculated, ensure that appropriate measures (i.e with UV and/or ozone) are made to disinfect water to ensure that only sanitary water is recirculated	The cost of installing and maintaining disinfection measures on recirculated water will be too costly for most business to voluntarily uptake this measure.	**Cost of fine filtration and UV disinfection (4mJ/cm²) of water:** **26 micron drum filter:** $30,000 **Drum screen annual service:** $2000 **UV unit:** $20,000 **UV globe annual replacemen**t: $3000
Minimisation of contact with potentially contaminated equipment	4	Access to quarantine zones is avoided where possible	The stakeholder raised concerns of the cost of purchasing multiple sets of equipment, some of which is expensive such as water quality meters. May be difficult for small farms with few staff members to allocate single staff member to perform biosecurity duties. It is possible for people to reduce access to quarantine areas where possible.	**Cost of purchasing dedicated equipment:** Nets: $400 Buckets: $56 Photometer: $2,100** Annual test kit reagent cost: $400** Water quality meters: $4,500

				Annual probe replacement cost: $400**
Detection of latently infected fish	5	None	The sensitivity of the diagnostic tests do not provide 100% exclusion of the pathogen (CyHV-3). Access to diagnostic laboratories is limited for CyHV-3. The cost of the diagnostic test is likely to make the stakeholder's business unviable. The stakeholder believes that he would not be able to see return on the investment as he is unable to reflect the cost back onto his customers.	**Cost of diagnostic tests:** Veterinarian to collect samples: $320 Travel: $500 PCR (single imported fish and thirty sentinels): $2,932.50 if all negative, $4,672.50 if suspect positives require confirmation. Sentinel koi: $150 Histology (30 sentinel fish): $4,500 Veterinary pathology interpretation: $160 **Cost to develop sampling protocols for koi farms:** Development of sampling protocols (7 days veterinary consulting): $10,500** Farm visits for implementation (4 farms, 1 day per farm): $9,000** Travel: $3,000**
Vaccination of existing stock	6	None	No vaccines available in Australia. Efficacy of vaccines is poor. The cost of vaccines and time required to individually inject each fish will be too exorbitant on the stakeholder's business.	**Research to develop efficacious vaccine and generation of data packages to prepare for an APVMA MUP application:** $750,000** **See**

			The business will not see any benefit on investment as market is unwilling to accept price increases.	**report on additional koi industry research needs.** **Cost to vaccinate fish:** Labour: $10,000 Vaccine table: $4,300 Vaccine guns: $1,200 Vaccine gun needles: $80 Anaesthetic (AQUI-S): $500 Off-label veterinary prescription: $120.00 Oxygen (Size E 4.1m^3): $305 Ceramic Airstones: $720 Water quality monitoring: $4,500 Annual probe replacement cost: $400** Vaccine: $1-3/fish
Prevention of spread of disease between systems/site and to another systems/site	7	In the event of a material disease in a pond/system, affected fish populations are culled. A decontamination process (i.e. hydrated lime (CaOH) application and drying of ponds in sunlight for a minimum of 7 days) must be undertaken prior to restocking. Decontamination must be undertaken in consultation with, or under instruction from, the relevant state government biosecurity	Culling of fish will likely be performed for affected populations (affected ponds/tanks only) only. Culling will likely result in the cessation of farming due to the loss of genetic lines. These lines are difficult to re-establish, and at times impossible due to availability in Australia.	**Cost to farmer:** Unrecoverable – business ending.

agency to ensure compliance with biosecurity obligations or with a competent veterinarian.

Fish movement is restricted for a minimum of one year (or until water temperatures fall below permissible ranges the following year). No new fish are permitted to be introduced into the site/fishery during the restriction period.

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility				
Risk activity	#	Feasible option	Concerns raised by stakeholders	Estimated cost
Management of effluent discharge	1	None	There are difficulties for the stakeholder to hold water without any other aquatic creatures such as mosquitofish, eels, frogs, turtles. The cost of large quantities of disinfectants is uneconomic for the stakeholder.	**Cost of decontaminating water:** Sodium hypochlorite 12.5% (to achieve 3mg/L) (90ML): $13,000** Cost of Chlorine photometer: $2,100** Annual test kit reagent cost: $400**
Appropriate disposal of mortalities	2	Fish mortalities are removed daily and disposed of by a method approved by the relevant authority, which ensures no risk of release of pathogens from the dead stock into waterways, or access for scavenger birds or animals (e.g. pigs, foxes, water rats) that could spread a disease.	The stakeholder disposes all mortalities by deep burial on site. No record keeping performed.	**Cost to collect and bury mortalities daily (0.5hr/d):** $7,300 **Cost to record mortalities daily (0.05hr/d):** $730
Management of people	3	None	Disinfection upon exit of the facility is possible, however it would likely be a deterrent for buyers and contractors which will go elsewhere to purchase fish. It would be unlikely that this would be adopted. The stakeholder believes that he would have a significant loss in revenue by	**Cost of implementing hand and footwear disinfection protocol:** **Chemical:** Ethanol (70%) (5L): $180** Virkon S (1kg): $100** Sodium hypochlorite 12.5% (20L): $100**

			implementing disinfection protocols for people on entry and exit.	**Equipment:** Hand spray bottle: $1.50** Footbath: $300** Disinfection tub: $50** **Cost to develop disinfection protocols for koi farms:** Development of customised disinfection protocols for koi farms (7 days veterinary consulting): $10,500** Field implementation of disinfection protocols (4 farms @ 1 day per farm): $9,000** Travel: $3,000**
Management of equipment	4	Dedicated equipment should be maintained for use exclusively on site. Dedicated equipment should not be removed from site and use for other purposes.	Stakeholder keeps dedicated equipment on the farm. No routine labelling is performed and is unlikely to be performed.	**Cost to label equipment:** Permanent marker pens: $5 Tape: $10 Labour to label equipment (1hr): $40

3.0 Approximate cost estimation of feasible biosecurity options for the hobbyist industry

Assumptions:

System volume: 10,000L

System area: ~25m^2

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

Risk activity	#	Feasible option	Concerns raised by stakeholders	Estimated Cost
Water source (and organisms entrained within) to farm/pond/tank.	1	Town-water is used wherever possible. For rural areas with limited access to town-water, bore-water is used instead.	Risk of potential failures at chlorination plants. The industry is seeking an assurance that this will not occur by demonstrating the effects of lower concentrations of chlorine on CyHV-3 at various concentrations of virus concentrations.	**Chlorine test kit:** $60 **Research project:** Effects of acute and chronic exposures of chlorine, avian crop and gut passage, and salinity to CyHV-3 infectivity $350,000 **See report on additional koi industry research needs.**
Disinfection/deactivation of CyHV-3 in incoming water.	2	None	Cost of UV likely out of reach of some hobbyists. The space required to hold large volumes of water is prohibitive. The level of understanding and knowledge to safely maintain and keep an ozone unit is beyond many keepers and introduces significant WHS risks. The stakeholders are wishing to understand the impact of salinity on CyHV-3 infectivity and viability. Further research will be required to further understand this aspect.	**Cost of UV disinfection (40W 4mJ/cm²):** $500 Annual globe replacement cost: $300

Management of water quality	4	Testing and recording of temperature, pH, TAN, nitrite, nitrate, algal bloom (visually) **weekly**. Maintain clean ponds.	Feasibility and adoptability only applicable for koi club members. Education is likely required for wider adoption (i.e water quality information guidelines published by the Government). There may be some resistance from some koi club members to perform routine water quality testing due to ongoing costs. Financial support for test kits may assist these members in adopting regular testing.	**Cost of weekly water testing:** **Manual titration Test kits:** $100 **Cost of education program:** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000 Workshop/seminar education dissemination/implementation for ~ 30 persons per 1 day workshop (4 days): $6,000 Venue hire (~4 days): $6,000 Catering: ~$4,000 Travel: $2,000 Film and edit workshops: $5,000 Cost of website and graphics design: $10,200 Cost of printout information sheets (2000 sheets): $770 Annual cost of maintaining server and website to host education guidelines: $1680
Prevention of potentially infected planktons, molluscs and	5	Use bore-water or town-water as the water source	The cost of chemical usage is likely to be cost prohibitive for the industry to treat unsanitary water sources such as riverine water. Risk of potential failures at chlorination plants was of	**Research project:** Effects of acute and chronic exposures of chlorine, avian crop and gut passage, and salinity to

crustaceans entry into ponds/tanks.			concern to the industry. The industry is seeking an assurance that this will not occur by demonstrating the effects of lower concentrations of chlorine on CyHV-3 at various concentrations of virus concentrations.	CyHV-3 infectivity $350,000** **See report on additional koi industry research needs.**
Prevention of wild carp entry into ponds/tanks.	6	Use of carp free water sources (bore-water or town-water) as the water source	Risk of potential failures at chlorination plants. The industry is seeking an assurance that this will not occur by demonstrating the effects of lower concentrations of chlorine on CyHV-3 at various concentrations of virus concentrations.	**Research project:** Effects of acute and chronic exposures of chlorine, avian crop and gut passage, and salinity to CyHV-3 infectivity $350,000** **See report on additional koi industry research needs.**
		Ensure adequate fencing/netting are in place to prevent bird access to ponds/tanks when appropriate.	It is not adoptable by the industry to erect netting around their ponds at all times as it is aesthetically unappealing. Some koi keepers design their backyards so that their ponds are the visual main feature. Members of the koi club can erect netting when predatory birds are more prevalent. Further research into the infectivity and viability of CyHV-3 from regurgitate/digested/faecal matter of birds that ingest infected carp/koi will assist in adoption of netting.	**Cost of bird nets to koi owners every 3 years per pond:** $700 **Research project:** Effects of acute and chronic exposures of chlorine, avian crop and gut passage, and salinity to CyHV-3 infectivity $350,000** **See report on additional koi industry research needs.**
Introduction of new koi onto premise	7	Only source fish from disease free facilities with known histories.	The only feasible and adoptable option for koi keepers is to source fish from disease free facilities with known histories. Vaccination and certifications are not viable for the industry. The current level of protection achieved by vaccination is poor for the industry. The current diagnostic procedures are not sensitive enough for sub-clinically infected, latent carriers of the disease for the industry to adopt the testing for certification. It was raised that certifications have been employed overseas with no success.	**Cost of diagnostic tests:** Veterinarian to collect samples: $320 Travel: $500 PCR (single imported fish and thirty sentinels): $2,932.50 if all negative, $4,672.50 if suspect positives require confirmation. Sentinel koi: $150

				Histology (30 sentinel fish): $4,500 Veterinary pathology interpretation: $160
Disease risk from newly introduced fish (quarantine)	8	None	Most koi keepers do not have access to and/or ability to quarantine koi. Quarantine is not a feasible option. Many koi keepers do not have the knowledge to recognise abnormalities even if there is access to quarantine facilities. Diseases such as KHVD may potentially be missed. Training and education may assist keepers that do have access to quarantine facilities to recognise diseases. For equipment, it is feasible and adoptable for koi club members to use dedicated equipment and disinfect when there are no other options with disinfectants such as sodium hypochlorite and Virkon S. Training, education on handling of chemicals and public awareness of risks associated with using potentially infected equipment will facilitate in the adoptability of this option.	**Cost of developing education program:** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Delivery of workshop/seminar (4 days): $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Annual cost of maintaining server and website to host education guidelines: $1680** **Cost to develop an education program:** Develop of education guideline for water quality, koi disease, sanitary

				practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Annual cost of maintaining server and website to host education guidelines: $1680**
Disease investigation/surveillance of sick/ freshly dead quarantined fish	9	None	The sensitivity of the current diagnostic methods is too low for the koi industry to adopt testing of sick/ freshly dead fish and for surveillance. The cost of diagnostic tests may impact the feasibility of the option. Further research into a more sensitive test that can be performed non-lethally and collected easily will assist in adoption of diagnostic testing.	**Cost of diagnostic tests:** Veterinarian to collect samples: $320 Travel: $500 PCR (three sick fish) $748 if all negative, $1200 if suspect positives require confirmation. Histology (3 sick fish): $450 Veterinary pathology interpretation: $160

Health status of new fish	10	Ensure that the health status of all new fish is adequate before stocking with existing stock.	The only feasible and adoptable option for the industry currently is to ensure that the health status of all new fish is adequate before stocking with existing stock. There is unlikely to be any adoption by the industry to perform diagnostic tests due to the sensitivity of current diagnostic methods. The cost of diagnostic tests may impact the feasibility of the option. Further research into a more sensitive test that can be performed non-lethally and collected easily will assist in adoption of diagnostic testing.	**Research project:** Improve sensitivity of diagnostic tests for sub-clinical CyHV-3 carriers. **See report on additional koi industry research needs.** **Cost of diagnostic tests:** Veterinarian to collect samples: $320** Travel: $500** PCR (single imported fish and thirty sentinels): $2,932.50 if all negative, $4,672.50 if suspect positives require confirmation.** Sentinel koi: $150** Histology (30 sentinel fish): $4,500** Veterinary pathology interpretation: $160** **Cost of physical examination of fish**: Veterinary exam: $320 Travel: $500 Veterinary report: $160

Reduction of risk associated with cohabitation/stocking of potential non-target species	11	Do not cohabitate potential non-target species which been described to potentially harbour the virus with koi.	It is feasible and adoptable for koi club members to not cohabitate potential non-target species (NTS) with koi. However, this may not be possible for non-koi club members. Public education may assist in adoption by general members of the public, but it is unlikely to be 100% effective.	**Cost to develop an education program (including risks of cohabitating other species with koi):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design (as add-on to other outlined materials): $2,000** Cost of printout information sheets (2000 sheets): $770** Annual cost of maintaining server and website to host education guidelines: $1680**
Reduction of access of vermin (i.e. birds, rodent) and pets/farm animals to koi ponds/ tanks/ associated infrastructure.	12	Erect bird fences to eliminate birds/pets from entering ponds. Control rodents by good feed storage and bait stations.	It is not adoptable by the industry to erect netting around their ponds at all times as it is aesthetically unappealing. Some koi keepers design their backyards so that their ponds are the main feature. Members of the koi club can erect netting when predatory birds are more prevalent. Further research into the infectivity and viability of CyHV-3 from regurgitate/digested/faecal matter of birds that ingest infected carp/koi will assist in adoption of netting.	**Cost of bird nets to koi owners every 3 years per pond:** $700

Minimisation of contact with potentially contaminated equipment	13	Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should not be brought into the facility. Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry. Disinfect all equipment with chemicals at doses stated in Table 6 after each use.	For equipment, it is feasible and adoptable for koi club members to use dedicated equipment and disinfect when there are no other options with disinfectants such as sodium hypochlorite and Virkon S. Training, education on handling of chemicals and public awareness of risks associated with using potentially infected equipment will facilitate in the adoptability of this option.	**Cost to develop an education program (including safe chemical handling and disinfection procedures):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Annual cost of maintaining server and website to host education guidelines: $1680**
Minimisation of risks associated with people/visitors/staff that may have been exposed to potentially contaminated water or diseased fish.	14	Visitors/ contractors that will come into contact with koi or associated equipment, structures or water must disinfect hands upon entry (and exit) to a koi facility, and between separate areas of facility	There is unlikely to be any adoption by koi keepers to disinfect hands prior to entry and exit of a koi facility each time. It is potentially feasible for koi keepers to enforce disinfection of hands for people that are wishing to touch fish or equipment. Visitors already park away from ponds and are accompanied by owners. These options are feasible and adoptable. It is not feasible or adoptable for koi keepers to enforce visitors to change into freshly laundered clothes or site provided clothing.	**Cost of Chemical:** Ethanol (70%) (5L): $180 Virkon S (1kg): $100 Sodium hypochlorite 5%: $25 **Cost of Equipment:** Hand spray bottle: $1.50

		Visitors must be accompanied by a staff member/owner at all times	Biosecurity declaration, signage, and visitor logbooks are unlikely to be kept unless legally required, and are not adoptable or feasible for the industry. It is unlikely that a biosecurity induction will be given to any visitors unless legally required. This is considered not feasible or adoptable for the industry.	Shallow plastic tub: $20
		Visitor vehicles to be parked in dedicated parking area, preferably remote to fish stock and fish movement loading area		
		Visitors are discouraged from touching walls tank/pond structures, water and/or fish		
Avoidance of feeding potentially contaminated food.	15	Feed sanitised food (i.e extruded pellets) only where possible. Do not feed materials that has been potential contaminated with CyHV-3.	It is not feasible for the industry to avoid all unsanitised food (i.e artemia, daphnia, bloodworms) during early life stages. Further research into the whether these organisms are infective carriers/ vectors (both biological and mechanical vectors) may assist koi keepers in assessing the risk of feeding these feed sources. For later life stages, extruded pellets are fed and it is feasible and adoptable for the industry to feed sanitised food.	**Cost to koi hobbyist dependent on research findings. See report on additional koi industry research needs.**
		Do not feed raw fish to koi.		

B: Risks for pathogen (CyHV-3) spread within a koi facility

B: Risks for pathogen (CyHV-3) spread within a koi facility				
Risk activity	#	Feasible option	Concerns raised by stakeholders	Estimated Cost
Minimisation of contact with potentially contaminated equipment	4	Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry.	There is a knowledge gap for koi keepers in handling and using chemicals safely, and training and education is likely required for adoption.	**Cost to develop an education program (including safe chemical handling):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Cost of maintaining server and website to host education guidelines: $1680**
Detection of latently infected fish	5	None	The sensitivity of the current diagnostic methods is too low for the koi industry to adopt testing for surveillance. The cost of diagnostic tests may impact the feasibility of the option. Further	**Cost of diagnostic tests:**

research into a more sensitive test that can be performed non-lethally and collected easily will assist in adoption of diagnostic testing. With the current test sensitivities, koi keepers are likely to perform visual infections on their fish without any diagnostic or veterinary input. Social media is likely to be used. As mentioned previously, there is a knowledge gap for koi keepers in recognising disease, potentially, diseases such as KHVD may be missed.

Veterinarian to collect samples: $320

Travel: $500

PCR (single imported fish and thirty sentinels): $2,932.50 if all negative,

$4,672.50 if suspect positives require confirmation.

Sentinel koi: $150

Histology (30 sentinel fish): $4,500

Veterinary pathology interpretation: $160

Cost to develop an education program (including recognising signs of disease):

Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000**

Workshop/seminar: $6,000**

Venue hire: $6,000**

Catering: $2,000**

Travel: $2,000**

Film and edit workshops: $5,000**

Cost of website and graphics design: $10,200**

				Cost of printout information sheets (2000 sheets): $770** Cost of maintaining server and website to host education guidelines: $1680**
Vaccination of existing stock	6	None	It is not feasible for owners to inject and handle fish multiple times. There is likely to be more adoption if the vaccine was given as an immersion bath or orally, however, the efficacy will likely have to be high (~99%) for at least 12 months before there is any adoption by the industry.	**Cost to vaccinate koi:** Veterinary prescription: $150 Vaccine cost: $15-30/fish Veterinary assistance: $150 Travel: $500
Prevention of spread of disease between systems/site and to another systems/site	7	In the event of a material disease in a pond/system, all fish are culled. For diseases caused by CyHV-3 koi keepers must report to relevant authorities. A decontamination process (i.e. hydrated lime (CaOH) application and drying of ponds in sunlight for a minimum of 7 days) must be undertaken prior to restocking. Decontamination must be undertaken in consultation with, or under instruction from, the relevant state government biosecurity agency to ensure compliance with biosecurity obligations or with a competent veterinarian. Fish movement is restricted for a minimum of one year (or until water temperatures	It is difficult to enforce culling for everyone especially if the fish are of high sentimental value. The koi clubs are wanting a compulsory reporting system and culling to be enforced by the government for CyHV-3 such that the koi clubs are able to enforce stricter biosecurity measures around affected populations. The koi club is likely to provide aid to any members whose entire collection is culled as a result of KHVD. There is likely to be a 12 month transport and trading restriction placed on the affected members (i.e not able to show any fish, not able to sell or trade any fish). For members whose collection is not completely culled, that member is likely to be excluded from all activities hosted by the club.	**Cost to koi club to assist members whose collection is affected by CyHV-3:** $1,500 **Cost to decontaminate pond(s):** Labour: $400 Chemicals: $200 **Cost of veterinary consultation:** $150 **Cost to state governments to maintain and enforce compulsory reporting and culling programs for CyHV-3 affected**

fall below permissible ranges the following year). No new fish are permitted to be introduced into the site/fishery during the restriction period.

populations and publish details at the individual level where disease is detected:

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility				
Risk activity	#	Feasible option	Concerns raised by stakeholders	Estimated Cost
Management of effluent discharge	1	Ensure that all effluent discharge and their associated aerosol are separate from intake water and supply.	It is feasible and adoptable for koi club members to ensure that their effluent discharge and aerosols are separate from their intake water source. For non-koi club members, it may be difficult for widespread adoption. Education programs may assist in wider adoption but is unlikely to achieve 100% efficacy.	**Cost to develop an education program (including safe, sanitary disposal of effluent water):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Cost of maintaining server and website to host education guidelines: $1680**

Appropriate disposal of mortalities	2	Fish mortalities are removed daily and disposed of by a method approved by the relevant authority, which ensures no risk of release of pathogens from the dead stock into waterways, or access for scavenger birds or animals (e.g. pigs, foxes, water rats) that could spread a disease.	Appropriate disposal of mortalities is feasible for koi club members. It is not feasible for koi club members to record mortalities. For non-koi club members, it may be difficult for widespread adoption. Education programs may assist in wider adoption but is unlikely to achieve 100% efficacy.	**Cost to develop an education program (including safe mortality disposal practice):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Film and edit workshops: $5,000** Travel: $2,000** Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Cost of maintaining server and website to host education guidelines: $1680**
Management of equipment	4	Dedicated equipment should be labelled and maintained for use exclusively on site. Dedicated equipment should not be removed from site and use for other purposes.	There may not be industry wide adoption of labelling as some people only have one set of equipment. Equipment are kept exclusively for koi use for koi club members. For non-koi club members, it may be difficult to get adoption of disinfection or for them to maintain equipment for dedicated use. Education programs may assist in wider adoption, but it is unlikely to achieve 100% efficacy.	**Cost to develop an education program (including, risks of using equipment used in koi ponds externally (i.e for fishing)):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000**

Workshop/seminar: $6,000**

Venue hire: $6,000**

Catering: $2,000**

Film and edit workshops: $5,000**

Travel: $2,000**

Cost of website and graphics design: $10,200**

Cost of printout information sheets (2000 sheets): $770**

Cost of maintaining server and website to host education guidelines: $1680**

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows

Risk activity	#	Feasible option	Concerns raised by stakeholders	Estimated Cost
Additional disinfection protocols at koi shows/auctions	1	For koi shows/auctions, additional disinfection with hydrogen peroxide based solution (i.e. Huwa-San©) at 60mg/L can be added into tanks, with care required in higher water temperatures (>20oC) if holding for longer than one hour	The option to add disinfectants such as Huwa-San © is feasible for the industry. Currently there is a knowledge deficit restricting the adoptability of this option. Research into the long and short term effects of disinfectants on koi will likely be required and deemed safe before there is any adoption by the industry. The current study only extends for an 8 hour duration which is inadequate for many koi shows as fish may be held on the site for up to 24 hours. Training will be required for koi keepers to understand how the chemical is handled and used safely.	**Cost to develop an education program (including safe chemical handling):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Cost of maintaining server and website to host education guidelines: $1680** **Cost to prepare current available data packages and identify areas where data**

				is unavailable for Huwa-San © to prepare for an APVMA MUP application: $40,000
Avoidance of certain show formats	2	Where possible, do not cohabitate koi or share water with koi from different systems/facilities/premises/ponds/tanks together with other koi. Where possible, adopt the English style layout for all koi shows to prevent risk of horizontal transmission of CyHV-3 through contact with infected fish.	It may be difficult to prevent members of the public from accidentally placing fish in wrong tanks/bins or fish from splashing water to adjacent tanks/bins. It may be feasible to add disinfectants such as Huwa-San © into tanks/bins to reduce those risks. Currently there is a knowledge deficit restricting the adoptability of this option. Research into the long and short term effects of disinfectants on koi will likely be required and deemed safe before there is any adoption by the industry. The current study only extends for an 8 hour duration which is inadequate for many koi shows as fish may be held on the site for up to 24 hours. Training will be required for koi keepers to understand how the chemical is handled and used safely. It is adoptable for the industry to install splash guards or obtain deeper tanks/bins to prevent/reduce risk of splashing or fish from jumping out, however, the feasibility of this option is restricted due to cost.	**Cost of deeper tanks at koi shows to prevent koi from jumping out of tanks (80 tanks 2m diameter 0.85m depth @ $1200 each):** $96,000 **Cost of splash guards to reduce water transmission at koi shows @ $100 each:** $8,000 **See report on additional koi industry research needs.** **Cost to develop an education program (including safe chemical handling):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design: $10,200**

				Cost of printout information sheets (2000 sheets): $770** Cost of maintaining server and website to host education guidelines: $1680**
Disinfection and disposal of transport water returning from auctions/shows	3	None	It is unlikely to be feasible for the whole industry to dispose of all waters from shows. It may be feasible for the industry to use disinfectants such as Huwa-San ©. Currently there is a knowledge deficit restricting the adoptability of this option. Research into the long and short term effects of disinfectants on koi will likely be required and deemed safe before there is any adoption by the industry. Training will be required for koi keepers to understand how the chemical is handled and used safely.	**Cost to develop an education program (including safe chemical handling):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000** Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Cost of maintaining server and website to host education guidelines: $1680**
Screening of koi prior to shows/auctions	4	Koi fish that visibly appear sick are not permitted to attend shows/auctions	It is feasible and adoptable for the industry to not permit fish that are visibly sick from attending shows/auctions. There may be limitations for koi club members in identifying diseases and a veterinarian may be required, however the cost of veterinary	**Cost of competent veterinary inspection and surveillance at koi shows/auction:** Travel: $1,000

			inspection and surveillance is likely to restrict the feasibility of the option at shows and auctions. The use of rapid detection kits such as lateral flow devices to detect CyHV-3 may be adoptable for the industry if it was sensitive for clinically affected and sub-clinically infected carriers of CyHV-3.	Time: $3,000 **Cost of rapid detection test kit (250 tests): $4,818 (may be as high as** $10,000 after likely import costs and mark-up for domestic supplier)
Limiting of koi permitted to shows/auctions	5	None	The koi clubs are seeking for compulsory reporting and culling of koi populations affected by CyHV-3. There is likely to be a one year movement and trade restriction applied to affected members by the koi club. For koi club members that have not culled their entire populations, a permanent movement restriction and trade restriction is likely to be enforced by the koi club for any koi club hosted activities.	**Annual cost/ loss of revenue to koi club from cessation of shows/ auctions due to emergence of CyHV-3:** $45,000 **Cost to state governments to maintain and enforce compulsory reporting and culling programs for CyHV-3 affected populations and publish details at the individual level where disease is detected:**
Reduction of transmission risks associated with equipment usage	6	Sharing of equipment such as nets is prohibited. Disinfect all equipment between use.	Currently, nets are labelled at shows and no sharing of equipment takes place. It is feasible and adoptable for the industry to implement disinfection protocols for equipment. Education and training regarding safe handling of chemicals is likely required for the option to be adoptable and feasible.	**Cost to develop an education program (including safe chemical handling):** Develop of education guideline for water quality, koi disease, sanitary practice (16 days veterinary consulting) $21,000** Workshop/seminar: $6,000** Venue hire: $6,000** Catering: $2,000** Travel: $2,000** Film and edit workshops: $5,000**

				Cost of website and graphics design: $10,200** Cost of printout information sheets (2000 sheets): $770** Cost of maintaining server and website to host education guidelines: $1680**
Reduction of cross contamination by people	7	Contact with water, equipment or fish during shows/auctions is discouraged (signs, physical barriers).	It is feasible and adoptable for the industry to discourage people from contacting water, fish and equipment during shows and auctions. However, it is unlikely to achieve 100% efficacy.	**Cost of additional signage and barriers at koi shows/ auctions:** $3,000 **Cost of designing signs to discourage contact with water, equipment or fish during shows/auctions:** $800

Acknowledgement

2018-190 National Carp Control Plan - Biosecurity Strategy for the Koi (*Cyprinus carpio*) industry is supported by funding from the FRDC on behalf of the Australian Government.

Conflict of interest

None.

Appendix 9 – Phase 5 Biosecurity plan

Biosecurity and Control strategies against Cyprinid Herpesvirus 3 (CyHV-3) for koi industry in Australia in relation to the potential release of the virus as a biocontrol agent.

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4 September 2019

Contents

1. Introduction

In recent years, CyHV-3 has been explored as a possible biocontrol measure for European carp in Australia. This virus has been detected in 33 countries including Canada, Indonesia, Japan, Taiwan, Thailand, Poland, Singapore, United Kingdom, and the United States of America, but at this time remains exotic to Australia.

Cyprinid Herpesvirus 3 (CyHV-3) also known as Koi Herpesvirus (KHV) is a notifiable disease known to affect koi (*Cyprinus carpio koi*) and European carp (*Cyprinus carpio*). CyHV-3 is the pathogen responsible for causing Koi Herpesvirus Disease (KHVD) which is the damaged tissues which result from viral replication. In subclinical infections with CyHV-3, no tissue damage may be evident, hence the fish is not technically 'diseased'. Such a fish is however an infected carrier of the virus which could potentially spread the virus to other susceptible fish.

The potential release of CyHV-3 into Australian waters would introduce new risks to Australia's koi industry. A literature review and biosecurity options list were compiled to assess and explore potential risk mitigation strategies against CyHV-3. An industry steering group stakeholder meeting in Sydney was held on 4th June2019 to explore and explain each biosecurity option identified from the literature review.

It was identified during the meeting by stakeholders that the risks and mitigation strategies against CyHV-3 differed vastly for koi hobbyists and koi farmers. As no koi farmers were at the initial meeting, FFVS reached out to current Australian koi farmers to participate in a teleconference. Only one koi farmer agreed to participate by teleconference. Further subsequent attempts to contact other koi farmers were unsuccessful. Hence the views in this aspect of the report may not reflect the adoptability of biosecurity options for the entire koi farming sector of the koi industry in Australia.

A further steering committee teleconference was held on the 25th June 2019 and the feasibility and adoptability of each option was discussed. Meeting minutes were distributed to relevant stakeholders following the meeting.

The feasible options identified by stakeholders are documented in the recommended biosecurity options. Some options have significant limiting factors which reduce the adoptability of the options for the stakeholders. Some of these factors include monetary, physical space constraints, knowledge/research, staffing, equipment and availability of registered products.

The risk rating in Table 5 assumes no mitigation is in place.

Sections 3 and 4 below outlines the range of risk mitigation options which were identified to be feasible and adoptable for each of the risk pathways(A-D) identified in the Table 5 Risk Assessment. The ratings (low, moderate, high, extreme) in the Mitigated Risk Rating column detail the qualitative residual risk of CyHV-3 outbreaks after applying the feasible option assuming there is 100% industry uptake.

The industry advised that it was unlikely that all options would be adopted by all koi keepers, even if the significant factors were removed, due to the diverse nature of the koi keepers who are not all members of koi clubs or the national associations.

2. Methods

2.1 Risk assessment matrix

Assessment and allocation of ratings for the likelihoods and consequences of each risk activity draw from the literature review in Phase 1. The risk rating is obtained through the methods described in Section 3. Biosecurity Risk Analysis of CyHV-3.

Risk activities were obtained from the literature review and from the document "Aquaculture Farm Biosecurity Plan generic guidelines and template" published by the Sub-Committee on Aquatic Animal Health of the Australian Government Department of Agriculture.

2.2 Risk mitigation options

A list of risk mitigation options were compiled from the literature review and the experience of the authors in Phase 1 of the project. These options were distributed to stakeholders and the feasibility and adoptability of the options were discussed in stakeholder meetings. Where additional options were not considered, stakeholders were invited to suggest potential risk mitigation measures to assist in improving biosecurity.

3. Biosecurity Risk Analysis of CyHV-3

This process helps identify the areas which require the greatest biosecurity investment to deliver maximum protection to the farm, or hobbyist pond and tanks, from incursion of CyHV-3 and disease impacts of Koi Herpesvirus Disease (KHVD).

Figure 5: Four steps of biosecurity risk analysis

3.1 Identify the hazards

In this project, the hazard being assessed is CyHV-3. Other hazards are outside the scope of the project but can be considered using this framework by koi owners and their veterinary advisors on another occasion.

CyHV-3 has been identified as a hazard because it has caused adverse impacts on koi health and production internationally. Additionally, this disease has not occurred in Australia (presently considered exotic), and should it be released as part of a carp control program, koi owners' / farmers' stock will be placed at an increased risk of exposure and impact.

3.2 Risk Assessment of hazards

To assign a level of risk to a hazard, two factors need to be determined – the likelihood of exposure on your site and the consequence(s) of it occurring on your site. Veterinarians with an interest in aquatic species will be able to assist with this section.

Likelihood can be estimated by considering the transmission pathways necessary for entry of a pathogen (disease causing agent-CyHV-3), and for exposure of your fish. For example, should CyHV-3 be released into the wild, the likelihood of exposure via water, when using water sources that contain wild carp will be 'certain', if the carp control program seeks to use the virus to control carp in all wild waters. If your facility is using chlorinated town water as the source, then the entry of CyHV-3 via this route, would be considered 'remote' as chlorination would be expected to deactivate the virus.

Similarly, pathways involving entry of infected (either clinical (expressing visual signs of disease) or sub-clinical (not exhibiting any obvious external visual signs of sickness)) live fish have the highest likelihood of causing exposure because they may shed the pathogen into your naïve, clean koi population and culture environment.

The likelihood rating for exposure will vary depending on:

- the properties of the pathogen
- the occurrence of the pathogen outside the site or in nearby sites and
- the possible pathways onto the site

Likelihood ratings and descriptors are shown in Table 1.

Rating	Descriptor
Remote (1)	Occurs less than once in 20 years
Unlikely (2)	Occurs not more than once in 5-20 years
Possible (3)	Occurs not more than once in 3-5 years
Likely (4)	Occurs not more than once in 2 years
Certain (5)	Occurs every year

Table 29**:** Assessment of disease likelihood

Consequence can be estimated by considering the impact(s) of the disease (where the pathogen has damaged tissues of the host fish) on the productivity/health of your fish population and enterprise. The consequences could include multiple aspects (e.g. mortality, reduced growth or food conversion, reduced product quality, reduced market access, lost sales, lost broodstock genetics, emotional stress and trauma from loss of a pet, decontamination costs, and/or treatment costs).

Consequence ratings and descriptors are shown in Table 2.

Rating	Descriptor
Insignificant (1)	Impact not detectable or minimal
Minor (2)	Impact is limited to some, not all, units and/or short term only
Moderate (3)	Impact of most populations on site, with increased mortality and/or decreased performance, but not business or hobby ending. Stock loss may result in some emotional stress.
Major (4)	All populations affected. Considerable impact resulting in serious supply constraints, stock loss and financial impact, some emotional trauma.
Catastrophic (5)	All populations affected. Likely complete depopulation of the site and possibly barriers to resumption of production/hobby, highly significant emotional trauma.

Table 30: Assessment of disease consequences

Risk estimation—Risk is estimated as a product of likelihood and consequence, resulting in risk ratings of 1–25. Risks are highest when both likelihood and consequence are high. However, the risk may be low even if the consequence is 'catastrophic', as the likelihood may be 'remote' for that particular circumstance; similarly, even if the likelihood is 'certain', the consequence may be 'insignificant'. Risk ratings can be determined by applying estimates of likelihood (where 1 is remote and 5 is certain) and consequence (where 1 is insignificant and 5 is catastrophic) to the risk matrix provided below in Table 3.

Likelihood rating / Consequence rating	Insignificant	Minor	Moderate	Major	Catastrophic
Remote	1	2	3	4	5
Unlikely	2	4	6	8	10
Possible	3	6	9	12	15
Likely	4	8	12	16	20
Certain	5	10	15	20	25

Table 31: Risk estimation matrix

The need for risk mitigation management responses flows from the risk estimation in Table 3 to the responses outlined in Table 4.

Risk level	Explanation and management response
1-2 Negligible	Acceptable level of risk. No immediate action required.
3-5 Low	Acceptable level of risk. On-going monitoring may be required.
6-10 Medium	Unacceptable level of risk. Active management is required to reduce the level of risk.
12-15 High	Unacceptable level of risk. Intervention is required to mitigate the level of risk.
16-25 Extreme	Unacceptable level of risk. Urgent intervention is required to mitigate the level of risk.

Table 32: Risk levels and management responses

Table 33: Risk assessment of koi industry activity

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

Risk activity	**Assumption**	**Likelihood**	**Consequence**	**Risk rating**
Water source (and entrained organisms in water) to farm/pond/tank	Surface waters that communicate with wild carp populations including areas connected only during flooding. Crustaceans, molluscs and planktons may potentially be carriers of CyHV-3 and transmit pathogens to koi.	Certain (5)	Catastrophic (5)	**Extreme (25)**
Introduction of new koi onto premise	High potential for some koi to be infected (sub-clinical carrier).	Likely (4)	Catastrophic (5)	**Extreme (20)**
Introduction disease from non-target species onto the facility/premise	Non-target species may become carriers of CyHV-3.	Possible (3)	Catastrophic (5)	**High (15)**
Introduction of disease from birds, vermin and pets/farm animals.	Regurgitated/digested CyHV-3 infected carp/koi may be infective. Birds, vermin and pet/farm animals may come into contact with CyHV-3 infected fish and/or contaminated material and bring infected material back to koi ponds/tanks.	Possible (3)	Catastrophic (5)	**High (15)**
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	People and equipment that have come into contact with water contaminated or fish infected with CyHV-3 may potentially be vectors for the disease	Possible (3)	Catastrophic (5)	**High (15)**
Introduction of disease (CyHV-3) through feed.	No feeding of raw fish. Only extruded pellets are fed. A slight potential that extruded feed could come into contact with a source of the virus.	Unlikely (2)	Catastrophic (5)	**Medium (10)**

B: Risks for pathogen (CyHV-3) spread within a koi facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Transmission of virus through aerosol/ water movement within a koi facility	Movement of the pathogen may occur via aerosol movement. Water which has not been treated may have come into contact with CyHV-3 infected fish which may allow for the virus to transmit to multiple tanks/ponds.	Likely (4)	Catastrophic (5)	**Extreme (20)**
Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.	People and equipment that have come into contact with water contaminated or fish infected with CyHV-3 may potentially be vectors for the disease. Equipment not dedicated to one fish group.	Possible (3)	Catastrophic (5)	**High (15)**
Movement of disease by birds, vermin, pets/farm animals between ponds/tanks	Diseased/latently infected fish are present on site.	Likely (4)	Catastrophic (5)	**Extreme (20)**
Spread of disease (CyHV-3) through feed.	Feed may potentially become contaminated with CyHV-3 and act as a vector for disease. Elevated opportunity for contamination of feed once virus is established on-site.	Possible (3)	Catastrophic (5)	**High (15)**

C: Risk to other koi facilities of release of CyHV-3 from an infected facility

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Discharge of pond water into storm water drainage	CyHV-3 may be present and viable in water from a pond containing infected koi (whether clinically, of sub-clinically infected).	Likely (4)	Catastrophic (5)	**Extreme (20)**
Discharge of pond water into wastewater (sewer) source	CyHV-3 may be present and viable in water from a pond containing infected koi (whether clinically, of sub-clinically infected).	Likely (4)	Insignificant (1)	**Low (4)**
Release of CyHV-3 through carriage of infected fish, or water which contains CyHV-3, by birds, vermin, pets/farm animals contacting infected	Birds, vermin, pets/farm animals have contact/access with the infected pond water and fish population.	Possible (3)	Catastrophic (5)	**High (15)**

site and moving to water bodies outside of the koi site.				
Disposal of mortalities	Fish mortalities are not promptly removed, buried, burnt, or bagged.	Possible (3)	Catastrophic (5)	**High (15)**
Release of the disease by people and equipment to wild riverine water source	People and equipment that have come into contact with water contaminated by, or fish infected with, CyHV-3 may potentially be vectors for the disease	Possible (3)	Catastrophic (5)	**High (15)**

D: Risks of pathogen (CyHV-3) spread at auctions and koi shows

Risk activity	Assumption	Likelihood	Consequence	Risk rating
Exposure through mixing of koi populations	Sub-clinically infected koi at shows/auctions will come into contact with uninfected koi. Purchasing of infected koi increases risk of spread of disease among existing stock at show, and upon return of stock to koi owner's site.	Certain (5)	Catastrophic (5)	**Extreme (25)**
Exposure to CyHV-3 through contaminated equipment and/or people	People and equipment will come into contact with water contaminated by, or fish infected with, CyHV-3.	Likely (4)	Catastrophic (5)	**Extreme (20)**

4. Hobbyist – Risk Management Options and Mitigated Risk Ratings

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

1) Water source (and entrained organisms in water) to farm/pond/tank

Mitigated Risk Rating	#	Risk Management Measures
Low	A1	Town-water is used wherever possible. For rural areas with limited access to town-water, bore-water is used instead.
Medium	A2	Testing and recording of temperature, pH, TAN, nitrite, nitrate, algal bloom (visually) **weekly**. Maintain clean ponds.
High	A3	Erect bird fences to eliminate birds/pets from entering ponds where appropriate. Control rodents by good feed storage and bait stations.

2) Introduction of new koi onto premise

Mitigated Risk Rating	#	Risk Management Measures
High	A4	Only source fish from disease free facilities with known histories.
Extreme	A5	Ensure that the health status of all new fish is adequate before stocking with existing stock.
Low	A6	Do not cohabitate potential non-target species which has been described to potentially harbour the virus with koi.

3) Introduction disease from non-target species onto the facility/premise

Mitigated Risk Rating	#	Risk Management Measures
Low	A7	Do not cohabitate potential non-target species which has been described to potentially harbour the virus with koi.

Introduction of disease from birds, vermin and pets/farm animals.

Mitigated Risk Rating	#	Risk Management Measures
Low	A8	Town-water is used wherever possible. For rural areas with limited access to town-water, bore-water is used instead.
High	A9	Erect bird fences to eliminate birds/pets from entering ponds where appropriate. Control rodents by good feed storage and bait stations.

4) Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.

Mitigated Risk Rating	#	Risk Management Measures
Medium	A10a	Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should not be brought into the facility. Disinfect all equipment with chemicals at doses stated in Table 6 after each use.

Medium	A10b	Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry.
Medium	A11a	Visitors/ contractors that will come into contact with koi or associated equipment, structures or water must disinfect hands upon entry (and exit) to a koi facility, and between separate areas of facility. Visitors are discouraged from touching walls tank/pond structures, water and/or fish.
Low	A11b	Visitors must be accompanied by a staff member/owner at all times.
Low	A11c	Visitor vehicles to be parked in dedicated parking area, preferably remote to fish stock and fish movement loading area.

5) **Introduction of disease (CyHV-3) through feed.**

Mitigated Risk Rating	#	Risk Management Measures
Medium	A12	Feed sanitised food (i.e extruded pellets) only where possible. Do not feed materials that has been potential contaminated with CyHV-3. Do not feed raw fish to koi.

B: Risks for pathogen (CyHV-3) spread within a koi facility

1) **Transmission of virus through fish movement and existing infected fish**

Mitigated Risk Rating	#	Risk Management Measures
Extreme	B0	No appropriate option was available. Further research is required. See Section 7.1.

2) **Transmission of virus through aerosol/ water movement within a koi facility**

Mitigated Risk Rating	#	Risk Management Measures
Low	B1	In the event of a material disease in a pond/system, all fish are culled. For diseases caused by CyHV-3 koi keepers must report to relevant authorities.
Low	B2	A decontamination process (i.e. hydrated lime ($Ca(OH)_2$) application and drying of ponds in sunlight for a minimum of 7 days) must be undertaken prior to restocking. Decontamination must be undertaken in consultation with, or under instruction from, the relevant state government biosecurity agency to ensure compliance with biosecurity obligations or with a competent veterinarian.
Low	B3	Fish movement is restricted for a minimum of one year (or until water temperatures fall below permissible ranges the following year). No new fish are permitted to be introduced into the site/fishery during the restriction period.

3) **Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.**

Mitigated Risk Rating	#	Risk Management Measures
Medium	B4	Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry (see Appendix 1).
Low	B5	Visitors must be accompanied by a staff member/owner at all times.

4) Movement of disease by birds, vermin, pets/farm animals between ponds/tanks

Mitigated Risk Rating	#	Risk Management Measures
High	B6	Erect bird fences to eliminate birds/pets from entering ponds where appropriate. Control rodents by good feed storage and bait stations.

5) Spread of disease (CyHV-3) through feed.

Mitigated Risk Rating	#	Risk Management Measures
Medium	B7	Feed sanitised food (i.e extruded pellets) only where possible. Do not feed materials that has been potential contaminated with CyHV-3. Do not feed raw fish to koi.

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility

1) Discharge of pond water

Mitigated Risk Rating	#	Risk Management Measures
Medium	C1	Ensure that all effluent discharge and their associated aerosol are separate from intake water and supply.

2) Release of CyHV-3 through carriage of infected fish, or water which contains CyHV-3, by birds, vermin, pets/farm animals contacting infected site and moving to water bodies outside of the koi site.

Mitigated Risk Rating	#	Risk Management Measures
High	C2	Erect bird fences to eliminate birds/pets from entering ponds where appropriate. Control rodents by good feed storage and bait stations.

3) Disposal of mortalities

Mitigated Risk Rating	#	Risk Management Measures
Low	C3	Fish mortalities are removed daily and disposed of by a method approved by the relevant authority, which ensures no risk of release of pathogens from the dead stock into waterways, or access for scavenger birds or animals (e.g. pigs, foxes, water rats) that could spread a disease.

4) Release of the disease by people and equipment to wild riverine water source

Mitigated Risk Rating	#	Risk Management Measures
Medium	C4	Dedicated equipment should be labelled and maintained for use exclusively on site.
Medium	C5	Dedicated equipment should not be removed from site and use for other purposes.

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows

1) Exposure to CyHV-3 through mixing of koi populations or through contaminated water

Mitigated Risk Rating	#	Risk Management Measures
Low	D1	For koi shows/auctions, additional disinfection with hydrogen peroxide based solution (i.e. Huwa-San©) at 60mg/L can be added into tanks, with care required in higher water temperatures (>20°C) if holding for longer than one hour
Medium	D2	Where possible, do not cohabitate koi or share water with koi from different systems/facilities/premises/ponds/tanks together with other koi.
Medium	D3	Where possible, adopt the English style layout for all koi shows to prevent risk of horizontal transmission of CyHV-3 through contact with infected fish.
Low	D4	Ensure that a disinfection protocol is applied to water transport trucks and associated equipment prior to filling tanks. If the disinfection status is unknown, disinfect water with a permitted chemical at dose rates listed in Table 6 (see Appendix 1).
Medium	D5	Koi fish that visibly appear sick are not permitted to attend shows/auctions.

2) Exposure to CyHV-3 through contaminated equipment and/or people

Mitigated Risk Rating	#	Risk Management Measures
Low	D6	Sharing of equipment such as nets is prohibited. Disinfect all equipment between use.
Medium	D7	Contact with water, equipment or fish during shows/auctions is discouraged (signs, physical barriers).

5. Farms

A: Incoming risks for pathogen (CyHV-3) entry onto a koi facility

1) Water source (and entrained organisms in water) to farm/pond/tank

Mitigated Risk Rating	#	Risk Management Measures
High	A1	Bore-water is used wherever possible. Where the use of bore-water is not feasible, lake/river/small dam waters are used with disinfection/ decontamination protocols applied.
High	A2	Testing when required: Dissolved oxygen (DO), Temperature, pH, Salinity, Total Ammonia Nitrogen (TAN), algal blooms to maintain good water quality. Maintain clean ponds.
Low	A3	Disinfect all incoming water with UV light at 4.0 x 10^3 µWs/cm^2 or apply ozone at 0.5mg/L total residual oxidants (TRO) concentrations of ozone for 15 seconds

2) Introduction of new koi onto premise

Mitigated Risk Rating	#	Risk Management Measures
Low	A4	Where a dedicated quarantine facility is not available, do not stock or introduce any new koi to the existing stock.
High	A5	Only source fish from disease free facilities with known histories.
Medium	A6	Quarantine all new koi upon arrival for a minimum of three (3) weeks
Medium	A7	Quarantine all new and returning fish with thirty (30) sentinel koi for the duration of the quarantine period
Medium	A8	Quarantine all new and returning fish upon arrival/return at permissive temperature for KHVD (16 °C – 28 °C).
Medium	A9	Observe quarantine fish daily for any abnormal observations.
Medium	A10	Monitor all new and returning fish in a dedicated quarantine system for signs of disease.
Medium	A11	All quarantine equipment remains in quarantine area. Where no alternative exists, and the use of non-dedicated equipment is required, non-dedicated equipment should be disinfected with chemicals described in Table 6 prior to use (see Appendix 1).
Medium	A12	Apply transport, temperature, handling and sampling stress to new and/or sentinel koi during the quarantine period.

3) Introduction disease from non-target species onto the facility/premise

Mitigated Risk Rating	#	Risk Management Measures
Medium	A13	Do not cohabitate potential non-target species which been described to potentially harbour the virus with koi.

4) Introduction of disease from birds, vermin and pets/farm animals.

Mitigated Risk Rating	#	Risk Management Measures

High	A14	Bore-water is used wherever possible. Where the use of bore-water is not feasible, lake/river/small dam waters are used with disinfection/ decontamination protocols applied to ensure that no carp/eggs can enter the system through intake water.
Medium	A15	Ensure adequate fencing/netting are in place to prevent bird access to ponds/tanks.

5) Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.

Mitigated Risk Rating	#	Risk Management Measures
Medium	A16	Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should not be brought into the facility. Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry (see Appendix 1).
Low	A17	Disinfect hands and footwear upon entry (and exit) to a koi facility, and between separate areas of facility
Low	A18	Staff/owners must wear freshly laundered clothes each day prior to entry into a koi facility.
Medium	A19	All visitors/contractors/researchers must be aware of the biosecurity required prior to being granted entry.
Low	A20	Visitors must be accompanied by a staff member/owner at all times
Medium	A21	Visitor vehicles to be parked in dedicated parking area, preferably remote to fish stock and fish movement loading area where possible.

6) Introduction of disease (CyHV-3) through feed.

Mitigated Risk Rating	#	Risk Management Measures
Low	A22	Feed sanitised food (i.e extruded pellets) only. Do not feed materials that has been potential contaminated with CyHV-3. Do not feed raw fish to koi.

B: Risks for pathogen (CyHV-3) spread within a koi facility

1) Transmission of virus through fish movement and existing infected fish.

Mitigated Risk Rating	#	Risk Management Measures
Extreme	B0	None

2) Transmission of virus through aerosol/ water movement within a koi facility

Mitigated Risk Rating	#	Risk Management Measures
Low	B1	Where water is recirculated, ensure that appropriate measures (i.e with UV and/or ozone) are made to disinfect water to ensure that only sanitary water is recirculated
High	B2	In the event of a material disease in a pond/system, affected fish populations are culled. A decontamination process (i.e. hydrated lime (CaOH) application and drying of ponds in sunlight for a minimum of 7 days) must be undertaken prior to restocking. Decontamination must be undertaken in consultation with, or under instruction from, the relevant state government biosecurity agency to ensure compliance with biosecurity obligations or with a competent veterinarian.
Low	B3	Fish movement is restricted for a minimum of one year (or until water temperatures fall below permissible ranges the following year). No new fish are permitted to be introduced into the site/fishery during the restriction period.

3) Transmission of disease from people and/or equipment such as nets, buckets, water testing equipment.

Mitigated Risk Rating	#	Risk Management Measures
Medium	B4	Access to quarantine zones is avoided where possible
Medium	B5	Equipment which has been in contact with fish or culture water external to the facility (including contractor equipment or plant), should not be brought into the facility. Dedicated equipment should be available for each system. If no alternative exists, then a thorough cleaning and disinfection with chemicals detailed in Table 6 must be performed prior to entry (see Appendix 1).
Low	B6	Disinfect hands and footwear upon entry (and exit) to a koi facility, and between separate areas of facility
Low	B7	Staff/owners must wear freshly laundered clothes each day prior to entry into a koi facility.
Low	B8	Visitors must be accompanied by a staff member/owner at all times

4) Movement of disease by birds, vermin, pets/farm animals between ponds/tanks

Mitigated Risk Rating	#	Risk Management Measures
Medium	B6	Ensure adequate fencing/netting are in place to prevent bird access to ponds/tanks.

5) **Spread of disease (CyHV-3) through feed.**

Mitigated Risk Rating	#	Risk Management Measures
Low	B7	Feed sanitised food (i.e extruded pellets) only. Do not feed materials that has been potential contaminated with CyHV-3. Do not feed raw fish to koi.

C: Outgoing risks for pathogen (CyHV-3) entry onto a koi facility

1) **Discharge of pond water**

Mitigated Risk Rating	#	Risk Management Measures
High	C0	None

2) **Release of CyHV-3 through carriage of infected fish, or water which contains CyHV-3, by birds, vermin, pets/farm animals contacting infected site and moving to water bodies outside of the koi site.**

Mitigated Risk Rating	#	Risk Management Measures
Medium	C1	Erect bird fences to eliminate birds/pets from entering ponds where appropriate. Control rodents by good feed storage and bait stations.

3) **Disposal of mortalities**

Mitigated Risk Rating	#	Risk Management Measures
Low	C2	Fish mortalities are removed daily and disposed of by a method approved by the relevant authority, which ensures no risk of release of pathogens from the dead stock into waterways, or access for scavenger birds or animals (e.g. pigs, foxes, water rats) that could spread a disease.

4) **Release of the disease by people and equipment to wild riverine water source**

Mitigated Risk Rating	#	Risk Management Measures
Medium	C3	Dedicated equipment should be maintained for use exclusively on site.
Medium	C4	Dedicated equipment should not be removed from site and use for other purposes.
Low	C5	Disinfect hands and footwear upon entry (and exit) to a koi facility, and between separate areas of facility
Medium	C6	Visitor vehicles to be parked in dedicated parking area, preferably remote to fish stock and fish movement loading area where possible.

D: Risks of pathogen (CyHV-3) entry at auctions and koi shows

The stakeholder does not attend koi shows or auctions. Shows and auctions are hosted by the hobbyist sector. Feasible options for this section are adopted from the feasible options outlined in Section 3D of this report.

1) Exposure to CyHV-3 through mixing of koi populations or through contaminated water

Mitigated Risk Rating	#	Risk Management Measures
Low	D1	For koi shows/auctions, additional disinfection with hydrogen peroxide based solution (i.e. Huwa-San©) at 60mg/L can be added into tanks, with care required in higher water temperatures (>20 ºC) if holding for longer than one hour
Medium	D2	Where possible, do not cohabitate koi or share water with koi from different systems/facilities/premises/ponds/tanks together with other koi.
Medium	D3	Where possible, adopt the English style layout for all koi shows to prevent risk of horizontal transmission of CyHV-3 through contact with infected fish.
Low	D4	Ensure that a disinfection protocol is applied to water transport trucks and associated equipment prior to filling tanks. If the disinfection status is unknown, disinfect water with a permitted chemical at dose rates listed in Table 6 (see Appendix 1).
Medium	D5	Koi fish that visibly appear sick are not permitted to attend shows/auctions

2) Exposure to CyHV-3 through contaminated equipment and/or people

Mitigated Risk Rating	#	Risk Management Measures
Low	D6	Sharing of equipment such as nets is prohibited. Disinfect all equipment between use.
Medium	D7	Contact with water, equipment or fish during shows/auctions is discouraged (signs, physical barriers).

6. Cautionary statement:

Through the process of consultation with stakeholders from industry it became apparent that not all of the biosecurity measures which had been identified from the literature review were considered practical and able to be implemented by the koi industry. The chosen measures were therefore not the most biosecure option identified by the literature review meaning that significant areas of risk are likely to remain.

Currently, industry wide adoption of quarantining is not possible. For many hobbyists, there is insufficient space and infrastructure to install a dedicated, biosecure, quarantine facility. The inadvertent introduction of latently infected, clinically normal koi into ponds/tanks remains a significant risk for the hobbyist industry, should CyHV-3 be released into open waters in Australia. Dedicated quarantine facilities were not available at the farm which participated in this project. Significant investment is required to erect dedicated quarantine facilities at koi farms.

No vaccinations for CyHV-3 are approved for use in Australia by the APVMA. Live attenuated vaccines have been used overseas and are reported to provide the highest level of protection. However, the use of live attenuated vaccines is not suggested in Australia due to the potential risk of spread of less virulent strains of CyHV-3 to wild carp populations thereby conflicting with the aim of the carp control program. Control over effluent discharge may not be possible for koi farms in all weather conditions. As such it remains plausible that a less virulent strain of the virus could be released into wild waterways. The stakeholders have highlighted that an efficacious vaccine would likely be used by hobbyists if available. The efficacy of killed vaccines was considered to be insufficient for the hobbyist industry to justify vaccinating their pet koi, even if they became commercially available. Further research and commercialisation of a more efficacious vaccine is suggested prior to the release of CyHV-3, if CyHV-3 is to be introduced into Australian waterways. By way of analogy, prior to release of calcivirus for rabbit control, an efficacious vaccine was available for hobbyist and commercial rabbit keepers.

Wildlife exclusion also poses a risk for the industry with the current proposed measures. Current use of bird nets is only limited to prevent bird predation when fish are smaller in both hobbyist and farm sectors. For koi hobbyist, bird nets are not always erected as it is visually unappealing, thus contrary to the reasons why hobbyist keep koi. In farms, the ongoing maintenance cost of erecting bird nets and the cost of the nets are uneconomic for farms. It has also been highlighted that exclusion of small birds is difficult for the farm. If CyHV-3 were to be released in Australia, further research into birds as potential vectors for CyHV-3 may assist in the understanding of this risk pathways for both farms and hobbyists.

Currently, both farmers and hobbyists have noted that disease investigations or disease screening (i.e PCR, histology, veterinary workup) are not adoptable for the industry due to factors including the diagnostic sensitivity and specificity of current tests for determining carrier fish status, cost of tests, and the availability and ease of access to diagnostic laboratories. Without disease screening and investigation, hobbyist and farmers can only speculate causes of disease events. Also, it has been highlighted by stakeholders that many koi hobbyist are unable to precisely recognise diseases or clinical signs of diseases. As such, there is significant risk of the disease disseminating to other populations unknowingly. The development of sampling methods which could determine sub-clinical carrier status with greater precision would be of value. The tests are already very sensitive at detecting the viral particles, however, in sampling fish non-lethally the presence of the virus can be missed.

The stakeholders have highlighted that non-koi club members will continue to pose significant risk to the koi industry as biosecurity information may not be readily available and easily distributed to said parties and uptake may be poor.

The likely efficacy of the combined biosecurity measures for an individual koi industry participant, who attends koi shows, or trades koi, is heavily contingent on a 100% uptake and full adoption of the biosecurity strategies. Such high levels of adoption in an industry with large numbers of hobbyists, is difficult/impossible to achieve in practice. Rigorous compliance monitoring may assist in improving uptake, however, would require significant recurring funding to maintain. Hence it will remain likely that some koi populations may become infected with CyHV-3 should the virus be released into Australia's wild waterways. Due to widespread fish movements within the koi industry, the inability for many koi keepers to quarantine koi, and the potential for movement of sub-clinical carriers, risk for unintended dissemination must be considered.

The greatest biosecurity protection that could be afforded to the koi industry is for the CyHV-3 release to not proceed, and for Australia to maintain its exotic disease status with respect to this pathogen.

7. Utility of the report

Should the CyHV-3 release proceed, these biosecurity measures will aid in providing increased biosecurity barriers for the koi industry against CyHV-3. Additionally, individual koi keepers and farms may also use the documents and previous biosecurity options detailed in Phase 1-3 of the project to identify areas where additional investment may facilitate in increasing biosecurity in ponds/ tanks/ farms.

General biosecurity practices suggested in this report may assist individual koi keepers and farms in preventing entry, spread and dissemination of diseases.

Additional research and education programs are likely to facilitate an improvement in biosecurity for koi keepers and farms against CyHV-3 and generally against other risks creating a lasting benefit.

8. Conclusion

The findings and results of Phase 1-5 of project 2018-190 can assist government in the final assessment of the potential release of the exotic pathogen, CyHV-3. There are key intrinsic factors of the koi industry which suggest the development and adoption of additional biosecurity measures will be challenging. Should the koi industry adopt the many biosecurity measures outlined, then it is likely a demonstrable reduction in risk would be achieved at the individual hobbyist and farm level. However, the risks from CyHV-3 are unable to be reduced to zero within the constraints for adoption identified by industry. These factors are detailed in Phase 2 and Phase 3 of the project. It is clear from the literature review and consultation with the industry that CyHV-3 would pose a significant risk to the koi industry even if all the outlined feasible biosecurity measures are adopted.

9.1 Hobbyist Emergency Response Plan Template

This template can assist a hobbyist in outlining the actions and responsibilities that are to be undertaken in the event that an emergency disease is suspected in the hobbyist pond/tanks. The text and tables below provide an outline which hobbyists can use to draft their own emergency response plan.

A. Define the Trigger to execute the plan

Terminology such as, "*unusually high, unexplained mortality*". This needs to be defined for the individual hobbyist, as the trigger may differ according to life stage or size e.g. daily mortality rate, abnormal stock behaviour, or certain clinical signs such as gill necrosis.

B. Important Contacts

Position	Name	Contact details
Koi keeper	Kohaku Koi	Mobile: 0499 999 999
		Phone: (07) 999 999
		Email: amber@example.com.au
Aquatic veterinarian	i.e "Matt Landos"	i.e "Mobile: 0437 492 863
		Email: matty.landos@gmail.com"
State Govt Aquatic Animal Health Officer		
Emergency Disease Watch Hotline		1800 675 888
State Govt Laboratory		

C. Responsibilities to notify and actions

Action	Person responsible to execute	Signature	Date
1. Contact aquatic veterinarian			__/__/__
2. Contact KSA/ KSWA			__/__/__
3. Contact neighbours with koi or NTS, or bodies who have received stock from the suspect affected pond(s)			__/__/__
4. Contact the relevant Government authority			__/__/__
5. Document and follow instructions as directed by Government authority			__/__/__

6. Halt all movement of koi and water from the pond(s)/tank(s) __/__/__
7. until disease status known and approval granted
8. Stop water movement out of the affected pond/tank __/__/__
9. Stop water movement out of the site, in the event of material disease. __/__/__
10. Collect typically affected sick koi and immediately submit for laboratory diagnostics with the assistance of an aquatic veterinarian __/__/__
11. Isolate any suspected disease stock from other stock on site __/__/__
12. Cease all non-essential visitor/contractor movements onto the site __/__/__
13. Cease movement of any equipment from suspect disease area to other areas. __/__/__
14. Restrict all non-essential movement into the suspect disease area. __/__/__
15. Compile a list of all movements of stock, people, equipment, feed, visitors and machinery in the previous 2 weeks __/__/__

D. Sample Collection, Packaging and Dispatch

Samples are to be collected by trained individuals, as advised by *the relevant authority*.

1. Sample collection

The following guidelines are to be followed when submitting fresh samples:

Seek advice from the state government Aquatic Animal Health Officer or aquatic veterinarian for collection and submission of samples.

- Do not sample already dead animals unless specifically requested to do so.
- Preferably, submit typically affected sick but still living koi.
- Where live samples cannot be readily moved to the laboratory, some samples should be preserved in 10% buffered formalin, some frozen in individual bags, and some freshly killed sick koi, sent on ice to the laboratory.
- Submit sick and healthy stock separately, in separate labelled pots.

2. Sample labelling

- Legible and permanent labelling of samples is required.
- A key list of samples should be sent to identify each sample in the package being sent to the laboratory
- Include the following information on a specimen advice form:
    - Site address
    - Contact details
    - Date
    - History of the event: when, where, which stock were affected

3. Packaging samples

- Samples must be carefully packed to avoid breakage, leakage or contamination. Multiple layers of sealed packaging must be used.
- Pack samples in an appropriate container (e.g. a disposable poly box or foam esky) together with sufficient paper or absorbent material to soak up any leakage. Secure the lid with tape and pack into a cardboard box.
- Use IATA 650 packing instruction. https://www.iata.org/whatwedo/cargo/dgr/Documents/packing-instruction-650-DGR56-en.pdf

4. Sample submission

Samples must be submitted as soon as possible following collection (particularly any fresh material on ice). Decomposed samples are of limited diagnostic value. Ring the laboratory to advise the shipment of samples is coming to them. Provide courier details if possible to allow tracking.

Submission details should include:

Name of Government laboratory

Address samples are to be submitted to

Contact number of laboratory liaison or case manager

Name and contact number of courier - transport may be arranged directly through the relevant authority or laboratory (ensure these arrangements are clear in this plan)

E. Disposal and Quarantine Protocols

Before implementing any disposal or quarantine protocols, instruction from the jurisdictional authority must be obtained to ensure compliance with General Biosecurity Obligations.

Insert disposal protocol information e.g. "Should this emergency plan be triggered mortalities will be rapidly collected using double-lined seafood bins (or other alternate). They will be transported to the approved onsite burial site, avoiding leakage enroute. No dead stock will be returned to the environment or accessible to scavengers".

Disposal options need consideration in this plan as to the volume of stock, based on farm size, which may be required to be disposed of. See AQUAVETPLAN – Operational procedures manual – Disposal www.agriculture.gov.au/SiteCollectionDocuments/animal-plant/aquatic/aquavetplan/disposal-manual.pdf *for further information.*

Insert details of quarantine protocols including isolation, disinfection etc. or reference a site-specific SOP on the subject of quarantine.

9.2 Farm Emergency Response Plan Template

This document needs to outline the actions and responsibilities that are to be undertaken in the event that an emergency disease is suspected in the farm. The text and tables below provide an outline which farms can use to draft their own emergency response plan.

A. Define the Trigger to execute the plan

Aquaculture farm licence conditions commonly use terminology such as, "*unusually high, unexplained mortality"*. This needs to be defined for the individual farm area, as the trigger may differ according to life stage or size e.g. daily mortality rate, abnormal stock behaviour, or certain clinical signs such as gill necrosis.

B. Important Contacts

Position	Name	Contact details
Farm Manager	Koi Fishy	Mobile: 0499 999 999 Phone: (07) 999 999 Email: fishyKoi@example.com.au
Nursery Manager		
Biosecurity Manager		
Logistics Manager		
Farm veterinarian	i.e "Matt Landos"	i.e "Mobile: 0437 492 863 Email: matty.landos@gmail.com"
State Govt Aquatic Animal Health Officer		
Emergency Disease Watch Hotline		1800 675 888
State Govt Laboratory		

C. Responsibilities to notify and actions

Action	Person responsible to execute	Signature	Date
16. Contact the Farm manager	*All staff should have capacity to elevate their concerns of a major disease outbreak*	_______	__/__/__
17. Contact farm veterinarian			__/__/__
18. Contact the relevant Government authority			__/__/__
19. Contact neighbouring farms, or bodies who have			__/__/__

received stock from the suspect affected farm

20. Contact KSA/ KSWA __/__/__
21. Document and follow instructions as directed by Government authority __/__/__
22. Halt all movement of koi and water from the farm until disease status known and approval granted __/__/__
23. Stop water movement out of the affected pond/raceway/tank __/__/__
24. Stop water movement out of the farm, in the event of material disease. __/__/__
25. Collect typically affected sick koi and immediately submit for laboratory diagnostics __/__/__
26. Isolate any suspected disease stock from other stock on farm __/__/__
27. Cease all non-essential visitor/contractor movements onto the farm __/__/__
28. Advise farm staff not to move any equipment from the suspect diseased area to other farm areas. __/__/__
29. Restrict all non-essential staff movement into the suspect disease area. __/__/__
30. Compile a list of all movements of stock, staff, equipment, feed, visitors and machinery in the previous 2 weeks __/__/__

D. Sample Collection, Packaging and Dispatch

Samples are to be collected by trained farm staff, as advised by *the relevant authority*, using the sampling SOP.

Document which staff members have been trained in sample collection and packaging.

1. Sample collection

The following guidelines are to be followed when submitting fresh samples:

Seek advice from the state government Aquatic Animal Health Officer for collection and submission of samples.

- Do not sample already dead animals unless specifically requested to do so.
- Preferably, submit typically affected sick but still living stock.
- Where live samples cannot be readily moved to the laboratory, some samples should be preserved in 10% buffered formalin, some frozen in individual bags, and some freshly killed sick koi, sent on ice to the laboratory.
- Submit sick and healthy stock separately, in separate labelled pots.

2. Sample labelling

- Legible and permanent labelling of samples is required.
- A key list of samples should be sent to identify each sample in the package being sent to the laboratory
- Include the following information on a specimen advice form:
    - Site address
    - Contact details
    - Date
    - History of the event: when, where, which stock were affected

3. Packaging samples

- Samples must be carefully packed to avoid breakage, leakage or contamination. Multiple layers of sealed packaging must be used.
- Pack samples in an appropriate container (e.g. a disposable poly box or foam esky) together with sufficient paper or absorbent material to soak up any leakage. Secure the lid with tape and pack into a cardboard box.
- Use IATA 650 packing instruction. https://www.iata.org/whatwedo/cargo/dgr/Documents/packing-instruction-650-DGR56-en.pdf

4. Sample submission

Samples must be submitted as soon as possible following collection (particularly any fresh material on ice). Decomposed samples are of limited diagnostic value. Ring the laboratory to advise the shipment of samples is coming to them. Provide courier details if possible to allow tracking.

Submission details should include:

Name of Government laboratory

Address samples are to be submitted to

Contact number of laboratory liaison or case manager

Name and contact number of courier - transport may be arranged directly through the relevant authority or laboratory (ensure these arrangements are clear in this plan)

E. Disposal and Quarantine Protocols

Before implementing any disposal or quarantine protocols, instruction from the jurisdictional authority must be obtained to ensure compliance with General Biosecurity Obligations.

Insert disposal protocol information e.g. "Should this emergency plan be triggered mortalities will be rapidly collected using double-lined seafood bins (or other alternate). They will be transported to the approved onsite burial site, avoiding leakage enroute. No dead stock will be returned to the environment or accessible to scavengers".

Disposal options need consideration in this plan as to the volume of stock, based on farm size, which may be required to be disposed of. See AQUAVETPLAN – Operational procedures manual – Disposal www.agriculture.gov.au/SiteCollectionDocuments/animal-plant/aquatic/aquavetplan/disposal-manual.pdf *for further information.*

Insert details of quarantine protocols including isolation, disinfection etc. or reference a site-specific SOP on the subject of quarantine.

10. Further Areas of Research and Development

10.1 Research

- The effects of salinity on CyHV-3 infectivity and persistence in the environment.
- The efficacy of free chlorine at concentrations below 3mg/L on CyHV-3 infectivity at various virus concentrations for varying durations.
- The effects of ozone on CyHV-3 infectivity.
- The infectivity and viability of CyHV-3 from regurgitate/digested/faecal matter of birds that ingest infected carp/koi.
- Development of a sensitive and specific sampling and testing protocol to detect infected koi sick koi
- Development of a sensitive and specific non-lethal sampling and testing protocol to detect latently, clinically normal infected koi.
- The infectivity (carrier/vector (mechanical and biological)) of artemia, daphnia, bloodworms to early life stages of koi.
- Development of a highly efficacious vaccine (~99%) that induces immunity for greater than one year against CyHV-3 that can be delivered by immersion bathe or oral ingestion.
- The efficacy and safety of Huwa-San© in a koi tank over a 24 hour period in preventing transmission of CyHV-3 to koi by cohabitation, aerosol, water transmission in transport stressed koi.
- Development of a pond side rapid detection test kit for latently infected, clinically normal koi.

10.2 Education programs

- Develop of education guideline for water quality, koi disease, sanitary practice
    - including risks of cohabitating other species with koi
    - including safe chemical handling and disinfection procedures
    - including safe chemical handling
    - including recognising signs of disease
    - including safe, sanitary disposal of effluent water
    - including safe mortality disposal practice
    - including, risks of using equipment used in koi ponds externally (i.e for fishing)

11. Acknowledgement

2018-190 National Carp Control Plan - Biosecurity Strategy for the Koi (*Cyprinus carpio*) industry is supported by funding from the FRDC on behalf of the Australian Government.

12. Conflict of interest

None.

Appendix 1: Table 6 – Disinfection dosages against CyHV-3

Disinfectant	Treatment time	Temperature (15°C)	Temperature (25°C)	Temperature (unspecified)
Iodophor (mg/L)	30 s	200	200	N/A
	20 min	200	200	N/A
Sodium hypochlorite solution (mg/L)	30 s	>400	>400	N/A
	20 min	200	250	N/A
Benzalkonium chloride solution (mg/L)	30 s	60	30	N/A
	20 min	60	30	N/A
Ethyl alcohol (%)	30 s	40	30	N/A
	20 min	30	25	N/A
Free Chlorine (mg/L)	30 min	N/A	N/A	3

Table 34 Chemicals used for disinfection of CyHV-3 adapted from Kasai et al. 2005.

FRDC FINAL REPORT CHECKLIST

Project Title:	Biosecurity strategies for the Australian Koi industry in relation to CyHV-3 and its potential release into Australian waterways		
Principal Investigators:	Chun-han Lin, Matthew A. Landos		
Project Number:	2018/180		
Description:	In 2019, Future Fisheries Veterinary Service developed a literature review and held two industry meetings to develop feasible biosecurity measures for the Australian koi industry against Cyprinid Herpesvirus 3 (CyHV-3), a significant disease of Koi and European carp.		
Published Date:	Not applicable	**Year:**	2019
ISBN:	Not applicable	**ISSN:**	Not applicable
Key Words:	Cyprinid Herpesvirus 3, European carp, koi, Cyprinus carpio, CyHV-3, KHV, KHVD, biosecurity		

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

	Is it included (Y/N)	**Comments**
Foreword (optional)	N	
Acknowledgments	Y	
Abbreviations	Y	
Executive Summary	Y	
− **What the report is about**	Y	
− **Background – why project was undertaken**	Y	
− **Aims/objectives – what you wanted to achieve at the beginning**	Y	
− **Methodology – outline how you did the project**	Y	
− **Results/key findings – this should outline what you found or key results**	Y	
− **Implications for relevant stakeholders**	Y	
− **Recommendations**	Y	
Introduction	Y	
Objectives	Y	
Methodology	Y	
Results	Y	
Discussion	Y	
Conclusion	Y	
Implications	Y	
Recommendations	Y	
Further development	Y	
Extension and Adoption	Y	

Project coverage	Y	
Glossary	N	
Project materials developed	Y	
Appendices	Y	

NATIONAL CARP CONTROL PLAN

The National Carp Control Plan is managed by the
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