Australian Government
Department of Agriculture

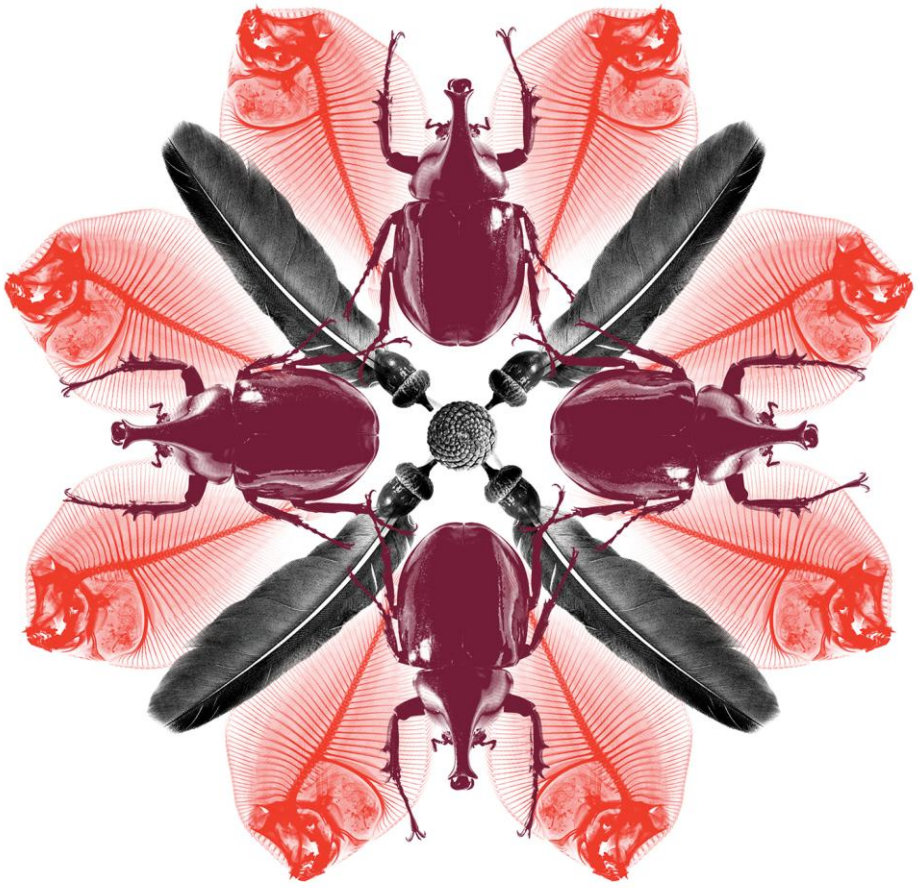
Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses

Final import risk analysis report

Biosecurity

Risk analysis reports

June 2014



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Cataloguing data

Australian Department of Agriculture 2014, *Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses—Final import risk analysis report*, Department of Agriculture, Canberra.

Internet

Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses—Final import risk analysis report is available at agriculture.gov.au/biosecurity.

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In 2005 researchers at the University of Sydney reported detection of an iridovirus considered exotic to Australia in several species of gouramis held at two Sydney pet shops. In experimental cohabitation trials, the virus was transmitted to Murray cod (*Maccullochella peelii peelii*), a freshwater fish species native to Australia. Murray cod is farmed as a foodfish and is listed as a threatened species (classified as vulnerable) under the *Environmental Protection and Biodiversity Conservation Act 1999* (EPBC Act 1999). The virus was also detected in clinically normal ornamental fish 28 days after experimental inoculation, indicating that some may be asymptomatic carriers for a period greater than the current combined pre-export and post-arrival quarantine period of 28 days.

In response to these findings, the Department of Agriculture advised that it would conduct a review of the policy on the importation of freshwater ornamental fish with respect to iridoviruses in March 2005. In September 2008, the Department of Agriculture announced that the policy review would be completed under the regulated import risk analysis (IRA) process as a standard IRA. The IRA was conducted in accordance with Australia's rights and obligations under the World Trade Organization (WTO) *Agreement on the Application of Sanitary and Phytosanitary Measures* (SPS Agreement) and is following the administrative steps set out in the Import risk analysis handbook 2011. As a standard regulated IRA, the process is to be completed within 24 months. A draft IRA report was released for a 60-day stakeholder comment period on 24 March 2009 (Biosecurity Australia Advice 2009/06). The consultation period was extended for 30 days on 21 May 2009 (Biosecurity Australia Advice 2009/12) until 24 June 2009 under regulation 69D of the Quarantine Regulations 2000. This final IRA report takes into account stakeholder submissions received on the draft report during the consultation period.

A 30-day appeal period for the provisional final IRA report commenced on the 22 July 2010. The Import Risk Analysis Appeals Panel (IRAAP) advised the Director of Animal and Plant Quarantine on the 7th October 2010 that all appeals had been either disallowed or found to be outside the ground for appeal.

As announced in Biosecurity Advice 2012/01, the then Director of Animal and Plant Quarantine decided to await the completion of a University of Sydney survey of Australian fish for gourami iridovirus before making a determination on the proposed final IRA. The department considers the findings of the survey report to be consistent with the assumptions in the IRA that wild fish and farmed food fish populations in Australia are free of the virus. Gourami iridovirus was not found in the limited populations of wild gouramis tested. As expected, the virus was detected

throughout the supply chain of imported ornamental fish (by importers, wholesalers and retailers) and in the progeny of imported fish in the single ornamental fish breeding facility to which the researchers had access. Although the department has monitored scientific developments since the release of the draft provisional final IRA report, new scientific information has not been added to this report, since it has not been of a kind that would change the IRA's conclusions.

This final IRA report assesses the biosecurity risks to Australia through the importation of freshwater ornamental fish with respect to iridoviruses (megalocytiviruses and ranaviruses) and examines risk management options to reduce risks to a level consistent with Australia's appropriate level of protection (ALOP).

The risk assessment concludes that importation of fish of the cichlid, gourami and poeciliid families, under the department's current import controls for freshwater ornamental fish do not achieve Australia's ALOP with respect to megalocytiviruses.

The report concludes that the current 14-day post-arrival quarantine period for gouramis and cichlids that targets iridovirus specific risks is not effective.

It is recommended that the families of the gourami – which includes fish of the subfamilies Luciocephalinae and Macropodinae of the family Osphronemidae, cichlid and poeciliid (for example, guppies, mollies, platyfish (platys) and swordtails) imported for ornamental purposes be permitted if fish are batch tested prior to export to show they are free of megalocytiviruses, or the fish are sourced from a country, zone or compartment that is recognised by Australia to be free of megalocytiviruses (based on active surveillance).

As a means of monitoring the effectiveness of overseas systems that underpin attestations about batch testing or country, zone or compartment freedom, it is recommended that imported shipments of cichlids, gouramis and poeciliids are subject to an on-going program of random post-arrival testing for megalocytivirus.

It is considered that these measures, in addition to existing pre-export quarantine measures would achieve Australia's ALOP with respect to iridovirus associated risks.

We have updated the IRA report to reflect stakeholder comments and new scientific information. The 2009 draft IRA report recommended tighter quarantine measures for ornamental fish for ranaviruses and megalocytiviruses. The final IRA report assesses these viruses separately and confirms risk management for megalocytiviruses is required while no risk management is required for ranaviruses.

Furthermore, for fish of the 'gourami family' (Osphronemidae), the draft IRA report recommended testing only for fish of the subfamily Luciocephalinae (fish normally referred to as 'gouramis'). Taking into consideration a report of megalocytivirus in paradise fish published after the completion of the draft IRA report, the final IRA recommends that megalocytivirus testing of the 'gourami family' be broadened to include fish of the subfamily Macropodinae, including Siamese fighting fish (bettas), paradise fish, licorice gouramis, pygmy gouramis and croaking gouramis.

This final report also differs from the 2010 provisional final IRA report in that it takes into account industry concerns about the commercial feasibility of post-arrival batch testing and recommends batch testing prior to export under the supervision of an approved competent authority.

The IRA report recognises that other measures may provide an equivalent level of protection against megalocytiviruses identified as being of quarantine concern. Submissions supporting equivalence measures will be evaluated on a case-by-case basis.

The diagnostic tests [for example, polymerase chain reaction (PCR) tests] used for active surveillance of source populations or batch testing must be appropriate for the purpose and adequately sensitive. Surveillance sampling must be consistent with the World Organisation for Animal Health (OIE) standard to detect the virus at a prevalence of 5 per cent with a confidence level of 95 per cent. PCR tests for diagnosing carriers (that is, animals infected but not showing clinical signs of disease) are available and it will be a matter for the laboratory undertaking the testing to acquire the appropriate technology.

A summary of the main changes in the final IRA report since release of the 2009 draft, are provided in Appendix A of this report.

1 Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious pests.

The risk analysis process is an important part of Australia's biosecurity policies. It enables the Australian Government to formally consider the risks that could be associated with proposals to import new products into Australia. If the risks are found to exceed Australia's appropriate level of protection (ALOP), risk management measures are proposed to reduce the risks to an acceptable level. But, if it is not possible to reduce the risks to an acceptable level, then no trade will be allowed.

Successive Australian Governments have maintained a conservative, but not a zero risk, approach to the management of biosecurity risks. This approach is expressed in terms of Australia's ALOP, which reflects community expectations through government policy and is currently described as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia's risk analyses are undertaken by the Department of Agriculture using technical and scientific experts in relevant fields, and involve consultation with stakeholders at various stages during the process.

The Department of Agriculture's assessment may take the form of an import risk analysis (IRA), a non-regulated analysis of existing policy, or technical advice.

Further information about Australia's biosecurity framework is provided in Appendix B of this report and in the Import risk analysis handbook 2011 on the Department of Agriculture website.

1.2 This import risk analysis

1.2.1 Background

Iridoviruses are reported to cause disease and mortality in a wide range of wild and farmed fish and amphibians, including freshwater ornamental fish – ornamental fish held in aquariums or ponds for sale, public or private display or for breeding. Iridoviruses known to be associated with freshwater fish in Australia are epizootic haematopoietic necrosis virus (EHNV) and lymphocystis disease virus (LCDV). EHNV has not been reported to occur in freshwater ornamental fish although experimental studies in Europe have shown that some species are susceptible via bath exposure (Ariel 2009).

Since Leibovitz and Riis (1980a) reported an acute systemic iridoviral disease in ram cichlids (*Mikrogeophagus ramirezi*)—formerly Ramirez' dwarf cichlids (*Apistogramma ramirezi*)—imported into the United States from South America, many iridoviruses causing systemic disease

in freshwater ornamental fish have been reported. The taxonomy of fish used in this review is as per FishBase, which is a web-based global information system on fish, accessed 26 March 2014.

With the exception of LCDV, no systemic iridoviruses of freshwater ornamental fish considered in this review are reported to occur in Australia and are therefore considered exotic. Iridoviruses from gouramis have previously been reported in Australia in freshwater ornamental fish under quarantine isolation prior to the implementation of the quarantine measures introduced in 1999 (Anderson et al. 1993)—if an exotic pathogen is reported during quarantine isolation, the consignment of imported ornamental fish is either destroyed or exported. Megalocytivirus was detected four times from fish held in post-arrival quarantine during 2000–04 as part of an ornamental fish testing project commissioned by the Department of Agriculture obtain data on disease occurrence during post-arrival quarantine (Stephens et al. 2009). An iridovirus associated with disease was also detected in farmed Murray cod (*Maccullochella peelii peelii*) in Victoria (2003), which was subsequently eradicated. The virus was later found by Go et al. (2006) to be a minor variant of dwarf gourami iridovirus (DGIV).

Researchers at the University of Sydney reported the detection of an iridovirus considered exotic to Australia in several species of ornamental gouramis held at two Sydney pet shops (Go et al. 2005; Go et al. 2006). The origin of the fish is unknown, but presumed to have been imported, suggesting that the current pre-export and post-arrival quarantine measures may be inadequate to manage risks associated with iridoviruses of quarantine concern. In experimental cohabitation trials conducted by the same researchers, the virus was transmitted to Murray cod, a farmed native foodfish. Murray cod is listed in Australia as a threatened species and classified as vulnerable under the *Environmental Protection and Biodiversity Conservation Act 1999* (EPBC Act 1999). Threatened species are further classified as extinct, extinct in the wild, critically endangered, endangered, vulnerable and conservation dependent. The virus was also detected in clinically normal gouramis 28 days after experimental inoculation (Go et al. 2005; Go and Whittington 2006). For gouramis and cichlids, Australia's current combined pre-export and post-arrival quarantine detention period is 28 days.

The *Import Risk Analysis on Live Ornamental Finfish* (Kahn et al. 1999), referred to in this report as 'the 1999 IRA', identified the need for specific risk management measures for all imported gouramis (subfamily Luciocephalinae of the family Osphronemidae) and cichlids (family Cichlidae) due to biosecurity risks associated with iridoviruses. However, the 1999 IRA considered the susceptibility of Australian native fish to iridoviruses of quarantine concern to be unknown, and no evidence available at the time that any threatened species in Australia would be affected. Further, the 1999 IRA did not identify farmed foodfish, which include Murray cod, as a group at risk from the introduction of iridoviruses. Except where 'finfish' is used in the title of documents such as the *Import Risk Analysis on Live Ornamental Finfish*, 'fish' will be used in this review as the general term unless otherwise stated.

The Department of Agriculture reviews import policies in the event of significant new scientific information. In response to the reported detection in Australia of exotic iridovirus in ornamental gouramis, the department announced on 11 March 2005 the review of its policy on the importation of freshwater ornamental fish with respect to iridoviruses (Animal Biosecurity Policy Memorandum (ABPM) 2005/01).

1.2.2 Transition into the regulated IRA process

Biosecurity Australia Advice 2008/29 announced on 11 September 2008, that the review of its policy on the importation of freshwater ornamental fish with respect to iridoviruses would be completed under the then new regulated IRA process as a standard IRA. The IRA is being conducted in accordance with Australia's rights and obligations under the WTO SPS Agreement and the Quarantine Regulations 2000, and is following the administrative steps set out in the Import risk analysis handbook 2011. As a standard regulated IRA, the process is to be completed within 24 months. A draft IRA report was released for a 60-day stakeholder comment period on 24 March 2009 (Biosecurity Australia Advice 2009/06). The consultation period was extended for 30 days on 21 May 2009 (Biosecurity Australia Advice 2009/12) until 24 June 2009 under regulation 69D of the Quarantine Regulations.

A provisional final IRA report was issued on 22 July 2010 for a 30-day period, during which period stakeholders who believed there was a significant deviation from the IRA process set out in the Import risk analysis handbook 2011 that adversely affected their interests could appeal to the Import Risk Analysis Appeals Panel (IRAAP). It is a non-judicial review that is not regulated under the Quarantine Regulations. Further details on the appeals process are set out in the Import risk analysis handbook 2011.

On 7 October 2010, the IRAAP advised the Director of Animal and Plant Quarantine that of the seven claims received it had disallowed six and found one claim outside the ground for appeal.

1.2.3 Scope of the IRA

The 1999 IRA covered all ornamental fish (both freshwater and marine), defined as those species included in Schedule 6, Part II of the then *Wildlife Protection* (*Regulation of Exports and Imports*) *Act 1982*, administered by the Australian Government Department of the Environment. This Act has since been superseded by the EPBC Act 1999, which refers to Part 1 of the 'List of specimens taken to be suitable for live import'—'Live specimens that do not require an import permit' (Permitted Species List). Those ornamental fish species in the Permitted Species List are unchanged, barring the addition of peacock gudgeon (*Tateurndina ocellicauda*), reticulate loach (*Botia lohachata*), humphead cichlid (*Cyphotilapia frontosa*), clown peckoltia (*Dekeyseria pulcher*), sawbwa barb (*Sawbwa resplendens*), dwarf botia (*Yasuhikotakia sidthimunki*) and red rainbowfish (*Glossolepis incisus*), and seahorses (*Hippocampus* spp.) being moved to Part 2 of the list.

This IRA report covers iridovirus risks associated with families of all freshwater ornamental fish species currently eligible for entry under the EPBC Act 1999. As such, it is not restricted to members of the gourami and cichlid families, but includes other families of freshwater ornamental fish (for example, Poeciliidae, Cyprinidae) in the Permitted Species List which are associated with iridoviruses of potential quarantine concern.

Due to the absence of more specific information, the 1999 IRA considered the biosecurity risk of freshwater ornamental fish associated iridoviruses as a whole (such as all freshwater fish iridoviruses as a single hazard). Information reported in scientific literature since 1999 sheds some light on the relationship between the various iridoviruses isolated from fish and the taxonomy of the Iridoviridae in general. This has allowed grouping of various iridoviruses based

on host taxonomy and treatment of each iridovirus group as a separate hazard for purposes of risk assessment.

Detailed information gathered in support of this assessment through a review of the scientific literature is provided in Chapter 4. This IRA report also documents the risk assessment and recommends risk management measures for the importation of freshwater ornamental fish with respect to megalocytiviruses.

1.2.4 Existing policy

The current quarantine measures for species of live freshwater fish permitted into Australia for use as ornamental fish (as announced in policy memorandum 1999/77) are established under the *Quarantine Act 1908* and are based on the 1999 IRA. The Department of Agriculture implements and administers the quarantine conditions.

The current conditions include that gouramis (subfamily Luciocephalinae) and cichlids are held in pre-export quarantine facilities approved by a competent authority recognised by the department for at least 14 days before export, health certification attesting that they are sourced from populations with no known significant clinical signs of disease in the previous six months, and that the fish are held in post-arrival quarantine for at least 14 days.

This IRA is a re-examination of iridovirus-associated risks (as determined in the 1999 IRA) taking into consideration further scientific information. Thus, the information in the 1999 IRA is essential to this analysis. Risk determinations in this analysis are based on information, assumptions and determinations made in the 1999 IRA, except where new information indicated the need for reassessment. Unlike the 1999 IRA, which based its unrestricted risk estimate on an assumption of nil quarantine controls on imported ornamental fish, this assessment's final 'unrestricted risk' estimation takes into account the quarantine measures currently in place for freshwater ornamental fish.

2 Method

2.1 Hazard identification and refinement

A pathogenic agent of quarantine concern was given detailed consideration in the 1999 IRA if it was assessed to be:

- carried by a Schedule 6 – currently known as the Department of the Environment List of Specimens Taken to be Suitable for Live Import– or related species of ornamental fish,
- infectious,
- exotic to Australia or present in Australia but subject to official control and
- listed by the World Organisation for Animal Health (OIE), or likely to cause significant harm if introduced into Australia.

The 1999 IRA dealt with iridoviruses of freshwater ornamental fish as a single hazard, primarily because of the absence at the time of data on taxonomic relationships between various isolates and species of fish they affect. Information that has become available since 1999 allows for a more detailed approach to hazard identification and risk assessment.

Table 1 provides details of iridoviruses isolated from fish and their proposed phylogenetic affiliations. The family Iridoviridae comprises of the genera *Iridovirus* (known to infect invertebrate hosts, so are not discussed further in this review), *Chloriridovirus* (known to infect invertebrate hosts, so are not discussed further in this review), *Lymphocystivirus*, *Megalocytivirus* and *Ranavirus* (Williams et al. 2000). The following iridoviruses are considered exotic to Australia, have the potential to cause significant disease if they were to establish or spread in Australia and are associated with families of freshwater ornamental fish species listed on the Australian Government Department of the Environment Permitted Species List.

Megalocytiviruses

Cichlids (fish belonging to the family Cichlidae):

- Angelfish iridovirus
- Cichlid iridovirus (includes ram cichlid and chromide cichlid). Although currently uncharacterised, based on histopathology cichlid iridoviruses are considered to be megalocytiviruses.
- Iridovirus in *Apistogramma* spp.
- Iridovirus in oscars
- Iridovirus in rainbow crib
- Iridovirus in curviceps

Gouramis (fish of the subfamilies Luciocephalinae and Macropodinae, family Osphronemidae):

- Dwarf gourami iridovirus

- Pearl gourami iridovirus
- Iridovirus in thick-lipped gourami, three-spot gourami and silver gourami
- Iridovirus in paradise fish

Poeciliids (fish belonging to the family Poeciliidae):

African lampeye iridovirus (all fish belonging to the subfamily Aplocheilichthyinae, family Poeciliidae)

- Swordtail iridovirus (although currently uncharacterised, based on histopathology swordtail iridovirus and other iridoviruses identified under the family Poeciliidae are considered to be megalocytiviruses)
- Iridovirus in mollies and platys
- Iridovirus in guppies

Ranaviruses:

- Poeciliids – Guppy virus 6 (GV–6)
- European catfish virus/European sheatfish virus (ECV/ESV) – now considered the same virus
- Cichlids – GV–6 and ECV/ESV
- Gouramis – GV–6 and ECV/ESV
- Zebrafish – GV–6 and ECV/ESV
- Goldfish – Rana tigrina ranavirus (RTRV) in goldfish

Uncharacterised iridoviruses:

- Goldfish – Goldfish iridoviruses 1 and 2 (GFV–1 and GFV–2).

Based on their phylogenetic relationships, these agents are grouped for the purposes of risk assessment as:

- Megalocytiviruses (ISKNV-like viruses)—in cichlids, gouramis and poeciliids
- Piscine ranaviruses—in poeciliids, cichlids, gouramis and zebrafish
- Amphibian ranaviruses—in goldfish
- Goldfish iridoviruses (GFV–1 and GFV–2)—in goldfish.

Table 1 Hazard identification and refinement

Taxonomic affiliation of fish iridoviruses			Exotic to Australia?	Iridovirus associated with families of freshwater fish species listed on the Department of the Environment Permitted Species List?	Potential to cause significant disease in Australia?	Retained for further risk assessment?
Genus	**Species**	**Isolates or strains within the species**				
Lymphocystivirus						
–	Lymphocystis disease virus 1					
–	–	Lymphocystis disease virus 1 (Chinchar et al. 2005)	No	Yes	No	No
–	Tentative species					
–	–	Lymphocystis disease virus 2 (Chinchar et al. 2005)	No	Yes	No	No
Megalocytivirus						
–	Infectious spleen and kidney necrosis like virus (freshwater) – ISKNV-like viruses					
–	–	Dwarf gourami iridovirusa (Do et al. 2005a; Go et al. 2006; F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009)	Yes	Yes	Yes	Yes
–	–	Iridoviruses in thick-lipped gourami, three-spot gourami pearl gourami and silver gourami (Go et al. 2006; Jeong et al. 2008b; Whittington et al. 2009)(F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009)	Yes	Yes	Yes	Yes
–	–	Iridovirus in paradise fish (Kim et al. 2010)	Yes	Yes	Yes	Yes
–	–	African lampeye iridovirus (Chinchar et al. 2005)	Yes	Yes b	Yes	Yes
–	–	Swordtail iridovirus (Paperna et al. 2001)	Yes	Yes	Yes	Yes
–	–	Iridovirus in mollies and platys (Paperna et al. 2001; F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009)	Yes	Yes	Yes	Yes
–	–	Iridovirus in guppies (Jeong et al. 2008b)	Yes	Yes	Yes	Yes
–	–	Cichlid iridovirus (Lewis and Leong 2004)[b] (F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009	Yes	Yes	Yes	Yes

Taxonomic affiliation of fish iridoviruses			Exotic to Australia?	Iridovirus associated with families of freshwater fish species listed on the Department of the Environment Permitted Species List?	Potential to cause significant disease in Australia?	Retained for further risk assessment?
Genus	**Species**	**Isolates or strains within the species**				
–	–	Iridovirus in oscars (Jeong et al. 2008a; Stephens et al. 2009; Whittington et al. 2009)	Yes	Yes	Yes	Yes
–	–	Iridovirus in *Apistogramma* spp. (Stephens et al. 2009)	Yes	Yes	Yes	Yes
–	–	Iridovirus in rainbow krib (Stephens et al. 2009)	Yes	Yes	Yes	Yes
–	–	Iridovirus in curviceps (F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009)	Yes	Yes	Yes	Yes
–	–	Angelfish iridovirus (Jeong et al. 2008b; Lewis and Leong 2004)	Yes	Yes	Yes	Yes
–	–	Infectious spleen and kidney necrosis virus (Chinchar et al. 2005)	Yes	No	Yes	No
–	Red Sea bream iridovirus (RSIV) like viruses (marine)					
–	–	Grouper sleepy disease iridovirus (Chinchar et al. 2005)	Yes	No	Yes	No
–	–	Flounder iridovirus (Do et al. 2005a)	Yes	No	No	No
–	–	Red sea bream iridovirus (Chinchar et al. 2005)	Yes	No	Yes (OIE listed)	No
–	–	Rock bream iridovirus (Do et al. 2005a)	Yes	No	No	No
–	–	Rockfish iridovirus (Do et al. 2005a)	Yes	No	No	No
–	–	Sea bass iridovirus (Chinchar et al. 2005)	Yes	No	No	No
–	–	Taiwan grouper iridovirus (Chinchar et al. 2005)	Yes	No	Yes	No
–	–	Turbot iridovirus (Do et al. 2005a)	Yes	No	No	No
***Ranavirus* – Piscine**						
–	Epizootic haematopoietic necrosis virus					
–	–	Epizootic haematopoietic necrosis virus (Chinchar et al. 2005)	No	Yes (experimental infection only)	Yes (OIE listed)	No
–	European catfish virus					

Taxonomic affiliation of fish iridoviruses			Exotic to Australia?	Iridovirus associated with families of freshwater fish species listed on the Department of the Environment Permitted Species List?	Potential to cause significant disease in Australia?	Retained for further risk assessment?
Genus	**Species**	**Isolates or strains within the species**				
–	–	European catfish virus/European sheatfish virus (Chinchar et al. 2005)	Yes	Yes	Yes[c] (Regionally listed OIE/NACA)	Yes
–	Santee-Cooper ranavirus					
–	–	Doctorfish virus (Chinchar et al. 2005)	Yes	No	Yes	No
–	–	Guppy virus (Chinchar et al. 2005)	Yes	Yes	Yes	Yes
–	–	Santee-Cooper ranavirus[d] (Chinchar et al. 2005)	Yes	No	Yes	No
–	Tentative species					
–	–	Singapore grouper iridovirus (Chinchar et al. 2005)	Yes	No	Yes	No
–	–	Pike-perch iridovirus (Tapiovaara et al. 1998)	Yes	No	Yes[e]	No
–	–	Short-finned eel ranavirus (Bovo et al. 1999)	No?	Yes	No	No
***Ranavirus* – Amphibian (OIE listed, chapter in preparation)**						
–	Bohle iridovirus					
–	–	Bohle iridovirus (Speare and Smith 1992)	No	Yes	Yes	No
–	Frog virus 3[f]					
–	–	Frog virus 3 (Granoff et al. 1965)	Yes	No	Yes	No
–	–	Tadpole oedema virus (Wolf et al. 1968)	Yes	No	No	No
–	–	Redwood Park virus (Mao et al. 1999)	Yes	No	No	No
–	–	Stickleback virus (Mao et al. 1999)	Yes	No	No	No
–	–	Tadpole virus 2 (Mao et al. 1999)	Yes	No	No	No
–	–	Lucke triturus virus 1 (Clark et al. 1968)	Yes	No	No	No
–	–	Rana temporaria United Kingdom iridovirus (Cunningham et al. 1996; Drury et al. 1995)	Yes	No	Yes	No
–	–	Bufo bufo United Kingdom virus (Cunningham et al. 2007b)	Yes	No	Yes	No

Taxonomic affiliation of fish iridoviruses			Exotic to Australia?	Iridovirus associated with families of freshwater fish species listed on the Department of the Environment Permitted Species List?	Potential to cause significant disease in Australia?	Retained for further risk assessment?
Genus	**Species**	**Isolates or strains within the species**				
–	–	Tiger frog virus or rana tigrina ranavirus (Kanchanakhan et al. 2003; Weng et al. 2002)	Yes	Yes	Yes	Yes
–	–	Bufo marinus Venezuelan iridovirus (Also known as Gutapo virus) (Zupanovic et al. 1998)	Yes	No	No	No
–	Ambystoma tigrinum virus					
–	–	Ambystoma tigrinum virus (Jancovich et al. 1997)	Yes	No	No	No
–	–	Regina ranavirus (Bollinger et al. 1999)	Yes	No	No	No
–	Unclassified ranaviruses					
–	–	Virus isolated from Leptodactylus spp. (Zupanovic et al. 1998)	Yes	No	No	No
–	–	Virus isolated from *Atelognathus patagonicus* (Fox et al. 2006)	Yes	No	No	No
–	–	Virus isolated from Rana catesbeiana (RCV–Z) (Majji et al. 2006)	Yes	No	Yes	No
–	–	Virus isolated from salamander (Hynobius nebulosus) (Une et al. 2009b)	Yes	No	No	No
–	Tentative species					
–	–	Rana esculenta iridovirus (Fijan et al. 1991)	Yes	No	Yes	No

Unassigned viruses[g] – Family Iridoviridae						
–	–	Erythrocytic necrosis virus (Chinchar et al. 2005)	Yes	No	Yes	No
–	–	White sturgeon iridovirus (Chinchar et al. 2005)	Yes	No	No	No
Uncharacterised fish iridoviruses						
–	–	Carp iridovirus (Lewis and Leong 2004)	Yes	Yes[h]	No	No
–	–	Eel iridovirus (Lewis and Leong 2004)	Yes	No	Yes	No
–	–	Goldfish iridovirus (Lewis and Leong 2004)	Yes	Yes	Yes	Yes

a An iridovirus outbreak associated with Murray cod in the State of Victoria in Australia (subsequently eradicated) was caused by a minor genetic variant of infectious spleen and kidney necrosis virus (ISKNV) and dwarf gourami iridovirus (DGIV). Thus, the virus associated with Murray cod is considered under DGIV. **b** Includes iridovirus in chromide cichlid and ram cichlid. **c** Fish of the family Siluridae not in Australia. ECV/ESV notifiable in Australian Capital Territory, South Australia and NSW only. **d** Santee-Cooper ranavirus (SCRV) is synonymous with largemouth bass virus (LMBV) and therefore will be used in this review document where SCRV or LMBV is reported in the literature. **e** Fish of the family Percidae (introduced species redfin perch now established in natural waters) are found in Australia. **f** Species listed on the IIIV ICTVB index of viruses (other than viruses associated with tortoises and turtles) are included here (Büchen-Osmond 2008). **g** The presence of large, non-enveloped virus particles in both assembly sites and paracrystalline arrays within the cytoplasm of infected cells is characteristic of iridovirus infections. Because of these distinguishing morphological features, several viruses infecting ectothermic animals have been tentatively identified as iridoviruses without further molecular or serological characterization. Furthermore, because many of these viruses have not yet been grown in culture little is known about their mode of replication and molecular organization (Büchen-Osmond 2008). **h** Although carp belong to the family Cyprinidae, they are prohibited entry into Australia. Thus, carp iridovirus is not retained for further risk assessment.

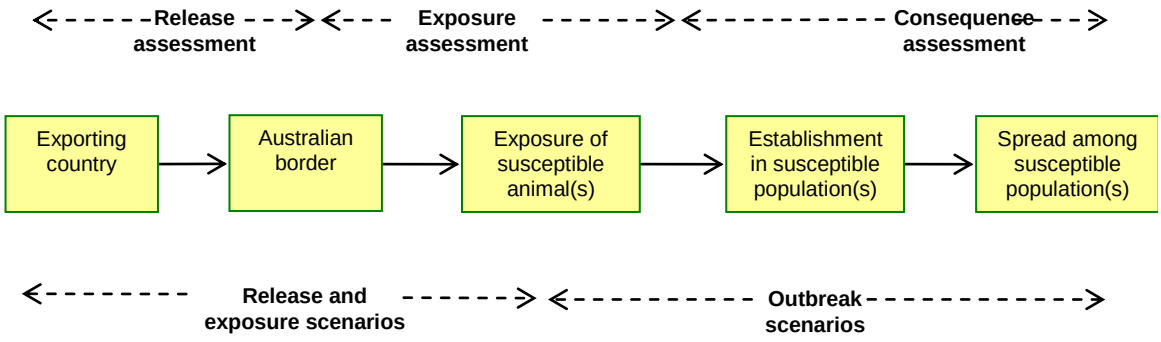
2.2 Risk assessment

Risk assessment is defined in the OIE Aquatic Animal Health Code (OIE 2009a) as the 'evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a hazard within the territory of an importing country'.

In accordance with the OIE Terrestrial Animal Health Code (OIE 2009b), the 'likelihood that a pathogenic agent will enter an importing country', and the 'likelihood that susceptible animals will be exposed to that agent', are determined in this IRA through a 'release assessment' and an 'exposure assessment', respectively. For the purposes of this review, 'release' will be used to mean 'entry of the agent', except in quotes from other documents. The 'likelihood of establishment or spread', and the 'biological, environmental and economic consequences of introducing a pathogenic agent', are determined through a 'consequence assessment'. The risk assessment for an identified agent concludes with 'risk estimation'—the combination of the likelihood of entry and exposure and likely consequences of establishment or spread—and yields the 'unrestricted risk estimate'. The 1999 IRA based its unrestricted risk estimate on an assumption of nil quarantine controls on imported ornamental fish. This assessment's 'unrestricted risk' estimation takes into account the quarantine measures currently in place for freshwater ornamental fish.

These general steps are illustrated in Figure 1. A more detailed schematic expanding on the main components of the release, exposure and consequence assessments is provided in Figure 2 (section 2.4).

Figure 1 Components of risk assessment



2.3 Evaluating and reporting likelihood

In common with other risk assessments on the importation of aquatic animals and their products, significant areas of knowledge are not covered in scientific literature. In this assessment, the department has used the available data, including information on related pathogenic agents and host species.

This assessment was conducted using a qualitative approach. The likelihood that an event will occur was evaluated and reported qualitatively, using qualitative likelihood descriptors for the release and exposure assessment, and the outbreak scenario (Table 2).

Table 2 Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition
High	The event would be very likely to occur
Moderate	The event would occur with an even probability
Low	The event would be unlikely to occur
Very low	The event would be very unlikely to occur
Extremely low	The event would be extremely unlikely to occur
Negligible	The event would almost certainly not occur

Likelihoods for the release and exposure assessment were combined using the matrix of 'rules' for combining descriptive likelihoods shown in Table 3.

Table 3 Matrix of 'rules' for combining descriptive likelihoods

Likelihood	High	Moderate	Low	Very low	Extremely low	Negligible
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate	–	Low	Low	Very. low	Extremely low	Negligible
Low	–	–	Very low	Very low	Extremely low	Negligible
Very low	–	–	–	Extremely low	Extremely low	Negligible
Extremely low	–	–	–	–	Negligible	Negligible
Negligible	–	–	–	–	–	Negligible

2.4 Risk assessment framework

The evaluation of disease risks resulting from the importation of freshwater ornamental fish involved estimating the likelihood of a susceptible host fish in Australia becoming exposed to an iridovirus of quarantine concern and the 'likely consequences' of such exposure.

In evaluating the likelihood of a susceptible host fish in Australia becoming exposed to a pathogenic agent of quarantine concern, the following factors were considered:

- the likelihood of the agent being released into Australia via fish being imported and released from quarantine detention (release assessment)
- in the event that the agent was released into Australia, the likelihood of susceptible host populations (representing one or more exposure groups) becoming exposed to the agent (exposure assessment).

Determination of 'likely consequences' required:

- identification of an outbreak scenario that could follow host exposure. (Although possible outbreak scenarios can range from no infection occurring to agent establishment or spread in a local population and further spreading to other susceptible host species only one likely outbreak scenario was assessed for each exposure group in this risk assessment)
- estimation of the likelihood of establishment or spread for that outbreak scenario
- impacts (biological, economical, and environmental) associated with that outbreak scenario.

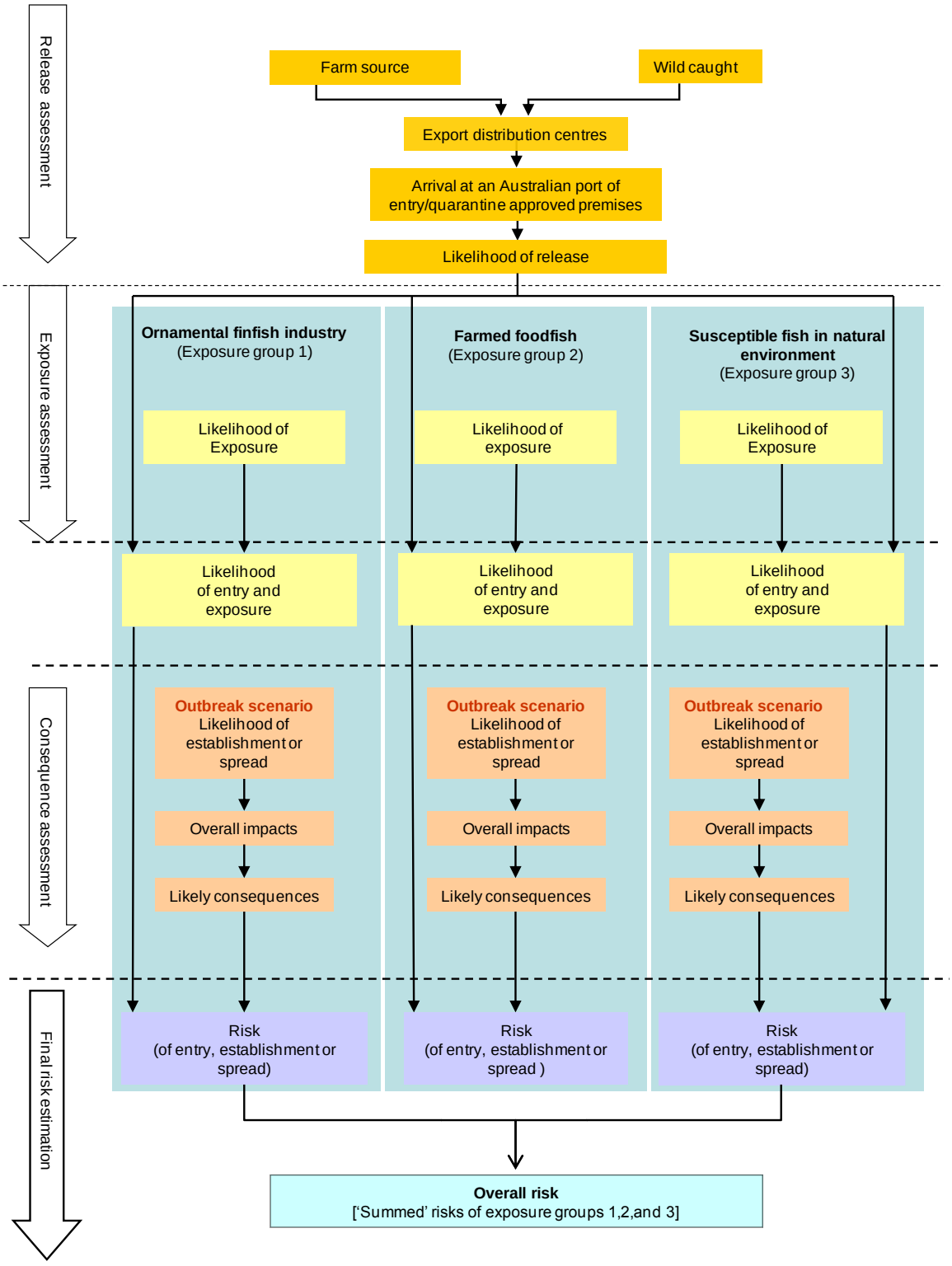
Likelihoods were assigned to release, exposure and establishment or spread (outbreak) scenarios as a whole. Likelihoods were not ascribed to individual pathway steps that make up each scenario. For example, in the exposure assessment there was a general examination of pathways whereby a susceptible host fish in an exposure group may become exposed to an agent. An overall likelihood of a susceptible host animal in the exposure group becoming exposed was then assigned.

The overall construct of this risk assessment, including the exposure groups identified is depicted in Figure 2.

This risk assessment looked at the likelihood of release and exposure of a hazard over a period of a year. As such, release and exposure assessments for each hazard took into consideration the estimated annual volume of trade in relevant species of freshwater ornamental fish. The subsequent consideration of the likelihood of establishment or spread and the impact assessment take into account events that might happen over a number of years even though only one year's volume of trade was considered. This difference reflects biological and ecological facts, for example where a pathogenic agent may establish in the year of import but spread may take many years.

Ornamental fish have been imported into Australia for many years. Accordingly, this assessment has taken this history of trade into consideration, including industry practices and the department's import controls to which imports have been subject prior to and since the 1999 IRA.

Figure 2 Elements of risk assessment



2.4.1 Release assessment

The release assessment considered a single release scenario, in which ornamental fish were sourced from farms (where fish were produced in outdoor earthen ponds or fibreglass or concrete tanks) or from the wild in the source country. On average 90 per cent of the world's freshwater ornamental fish are farmed and 10 per cent are collected from the wild (Olivier 2001).

Generally, ornamental fish farm stocks are checked for visible external parasites and clinical signs of disease prior to harvesting to ensure that fish are healthy and fit for transportation. Only visibly healthy fish are moved to holding tanks for sorting (for example, size, male/female) and counting. Fish showing clinical signs of disease may undergo treatment and be held on the farm until they recover and are fit for transportation.

Feeding is usually withheld for up to 72 hours prior to shipping, depending on the species, to provide adequate purging time. The fish are then counted again, packaged into batches (the number of fish per batch depends on species, size and the customer order) and held in individual aquariums, trays, buckets or other containers. At this step, it is possible that ornamental fish from different ponds or sources are mixed. Cross contamination can also occur due to unclean equipment that is shared across batches.

Fish are placed in polythene bags approximately one third filled with fresh water in preparation for transport. The bags are inflated with pure oxygen (two thirds), sealed with rubber bands or clips and placed in polystyrene boxes or cartons fitted with a plastic lining and then sealed. There may be one or more bags per box, but each bag is required to contain only one species of fish. Each box or carton is then labelled and individually identified.

Once packaged, the fish are transported to wholesalers or export distribution centres where the fish may be unpacked and held in a holding facility for conditioning/stabilisation to ensure they are fit for export. An export distribution centre may be located in a different country from where the fish were farmed or collected from the wild. Any fish showing clinical signs of disease or visible presence of parasites are treated while being acclimatised in the holding facility or disposed. Acclimatization is the process of slowly introducing the fish to different quality water to allow physiological adjustments to occur gradually over time.

Once the bags are unloaded at the final packaging room, the water is again changed and the bags re-oxygenated. Fish for export are packaged as described earlier for transportation in sealed polythene bags in insulated cardboard boxes.

In the wholesale or export distribution centre holding facilities, fish from different sources may again be mixed prior to export to meet customer orders. Cross contamination of fish from different batches may occur in these facilities due to inadequate cleaning and disinfection of equipment between batches.

In export distribution centres, ornamental fish destined for Australia are subject to quarantine isolation and visual inspection (in accordance with current import conditions)

and exported to Australia accompanied by a health certificate provided by the competent authority of the exporting country.

On arrival at an Australian port, ornamental fish are inspected by a Department of Agriculture officer and then ordered into post-arrival quarantine for one to three weeks, depending on species.

The likelihood estimation of the release assessment includes the current pre-export and post-arrival risk management measures for the importation of ornamental fish.

The final outcome of the release assessment is the likelihood of release of the agent into Australia, up to and including the point that fish are released from post-arrival quarantine.

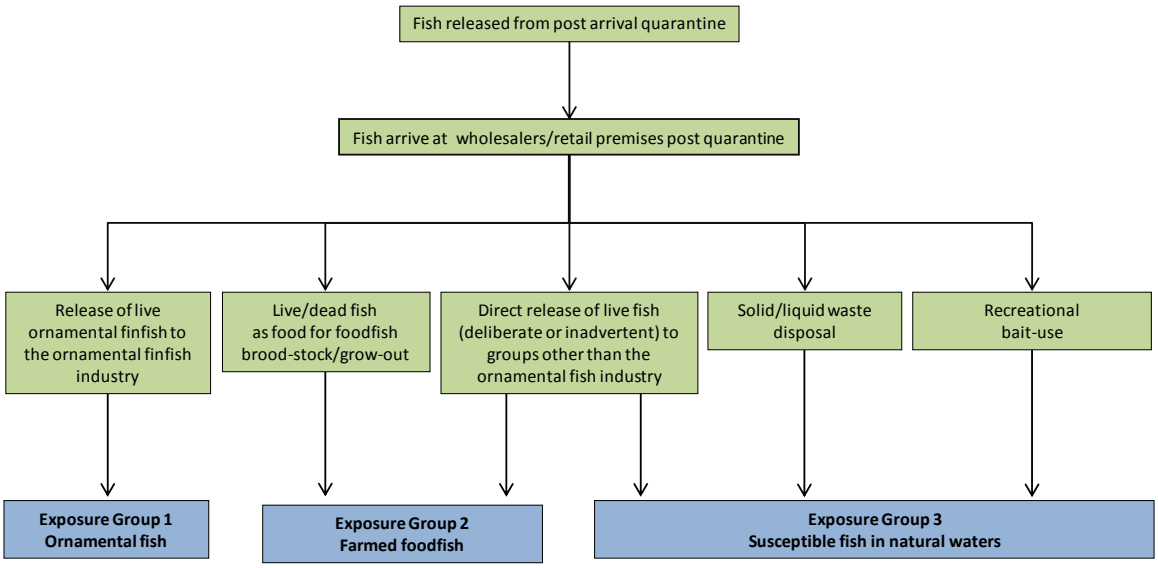
2.4.2 Exposure assessment

If released from post-arrival quarantine, the vast majority of imported fish are transferred to wholesale holding facilities, after which they are generally sold to retailers (that is aquarium shops or pet shops) where imported fish may or may not be mixed with locally bred fish.

From retailers, most fish are sold into home aquariums or ponds and a very small number as broodstock to commercial and 'backyard' breeders, or as exhibits in public aquariums. Some imported ornamental fish may be diverted to other end-uses (that is bait or fish food) so that the examination of exposure pathways in this assessment considers: the exposure of fish in natural waters via direct release of live imported ornamental fish by hobbyists; disposal of wastewater and dead fish, the use of imported ornamental fish as bait in recreational fishing; and the use of imported ornamental fish as food in the foodfish aquaculture industry.

Some imported ornamental fish such as goldfish (*Carassius auratus auratus*) and poeciliids may be kept in outdoor open ponds, from which they could escape to natural waters and thereby potentially expose susceptible host species in natural waters to exotic pathogenic agents Figure 3.

Figure 3 Potential exposure pathways



The exposure assessment considers the key distribution pathways and end-uses leading up to the potential exposure of the following three exposure groups, and determines the likelihood of exposure for:

- fish populations within the ornamental fish industry – includes ornamental fish wholesalers, retailers, breeders (commercial, semi-commercial and backyard), hobbyists and public aquariums
- farmed foodfish populations
- susceptible host species in natural waters.

The farmed foodfish exposure group includes fish grown for human consumption in ponds, raceways, cages and tanks, as well as foodfish species kept in research facilities and government hatcheries.

Use of imported live ornamental fish as broodstock or fingerlings for foodfish aquaculture operations was not considered in this assessment—diversion of imported ornamental fish in this way was assumed not to occur (as a basic premise) in the 1999 IRA. The department would need to reconsider the risk with importation in the event that such diversions were likely to occur, as was done with respect to humpback grouper (*Chromileptes altivelis*), commonly referred to as barramundi cod in Australia (ABPM 2004/18).

For each iridovirus of quarantine concern, the final outcome of the exposure assessment is an estimate of the likelihood that a domestic population of susceptible host species is exposed to potentially infected ornamental fish and/or associated contaminated materials imported into Australia. Estimation of likelihood of exposure also takes into consideration the relative volumes of potentially infected live ornamental fish and/or contaminated materials likely to be directed toward each exposure group.

Detailed data on relative volumes of live ornamental fish directed towards each exposure group are unavailable. Based on a report by the PSM Group Pty Ltd (1999) an estimated 95 per cent of imported ornamental fish are directed towards the ornamental fish industry. The report indicated that wholesalers import around 80 per cent of the total volume of freshwater ornamental fish with approximately 87 per cent sold to retailers, 7 per cent to other wholesalers and 3.5 per cent to hobbyists. Retailers import the remaining 20 per cent and sell 99 per cent directly to hobbyists and less than 0.1 per cent to commercial breeders.

A report by O'Sullivan et al. (2008) indicates that in 2006–07, over 80 per cent of freshwater ornamental fish purchases by wholesalers were imported for wholesale primarily to retailers. Retailers imported the remainder directly. Retailers sold 99 per cent directly to hobbyists and less than 1 per cent to other retailers (includes both imported and locally produced fish). Commercial breeders purchased more than 60 per cent of their fish from imported stocks. They sold around 50 per cent of their fish to retailers, around 47 per cent to wholesalers and less than 1 per cent to hobbyists (includes both imported and locally produced fish). Commercial ornamental fish breeders are those that breed and sell ornamental fish from a facility with a total water holding capacity of more than 10 000 litres (O'Sullivan et al. 2008).

2.4.3 Estimation of the likelihood of entry and exposure for each exposure group

The likelihood of entry and exposure is the exposure-group specific likelihood that there will be at least one host exposure event during a year. This likelihood was determined for each of the three exposure groups—ornamental fish industry, farmed foodfish and susceptible host species in natural waters.

The likelihood of entry and exposure for each exposure group was estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of 'rules' for combining descriptive likelihoods (Table 3 in Section 2.3).

The estimation of the likelihood of entry and exposure took into consideration the volume of product to be imported during a prescribed period. The period chosen by the department is one year, which was considered a sufficient period to enable evaluation of seasonal effects. Based on data from one major wholesale importer, of the estimated 12.5 million freshwater ornamental fish imported into Australia in 2003–04, approximately 57 per cent comprised poeciliids, 25 per cent goldfish, 8 per cent catfish, 8 per cent gouramis and 2 per cent cichlids. Department of Agriculture data shows that approximately 19 million ornamental fish were imported into Australia in 2008.

2.4.4 Consequence assessment

Criteria for assessing consequences associated with a pest or disease incursion are outlined in the relevant Australian legislation and international agreements, and in the standards prepared by the OIE. In particular:

- The *Quarantine Act 1908* and Proclamation 1998 as amended require decision-makers to take into account the level of quarantine risk, which means the probability of a disease or pest being introduced, established or spread in Australia, the Cocos Islands or Christmas Island and causing harm to humans, animals, plants, other aspects of the environment, or economic activities), and the probable extent of the harm (Section 5D).

- The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) Article 5 states that 'Members shall take into account as relevant economic factors: the potential damage in terms of loss of production or sales in the event of entry, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing Member; and the relative cost-effectiveness of alternative approaches to limiting risks.'

The OIE expands the 'relevant economic factors' described in the SPS Agreement and provides examples of factors that will typically be relevant to an IRA. In each case, consequence assessments do not extend to considering the benefits or otherwise of trade in a given commodity, nor to the impact of import competition on industries or consumers in the importing country.

The OIE Terrestrial Animal Health Code (OIE 2009b) also states that a consequence assessment should 'describe the potential consequences of a given exposure and estimate the probability of them occurring'. This approach is reflected in Quarantine Proclamation 1998, which requires that the 'level of quarantine risk' (which is defined under section 5D of the Quarantine Regulations to include the 'probable extent of the harm') is considered in making quarantine decisions.

Estimation of 'likely consequences' is addressed in terms of direct and indirect impacts on animal and plant life and health on a national scale, including biological, economic and environmental effects, and separately in terms of consequences to human life or health (if applicable). The latter is dealt with separately because primary responsibility for matters of human life or health rests with other government agencies and not the Department of Agriculture.

The following steps were taken to assess the 'likely consequences' associated with iridovirus entry and exposure:

1. Identification of a likely outbreak scenario that may occur as a result of release of an iridovirus of quarantine concern and host exposure to that iridovirus.

2. Estimation of the likelihood of that outbreak scenario occurring to obtain a likelihood of establishment or spread for the outbreak scenario.

3. Determination of the level and magnitude of adverse (biological, economic, and environmental) impacts resulting from that outbreak scenario.

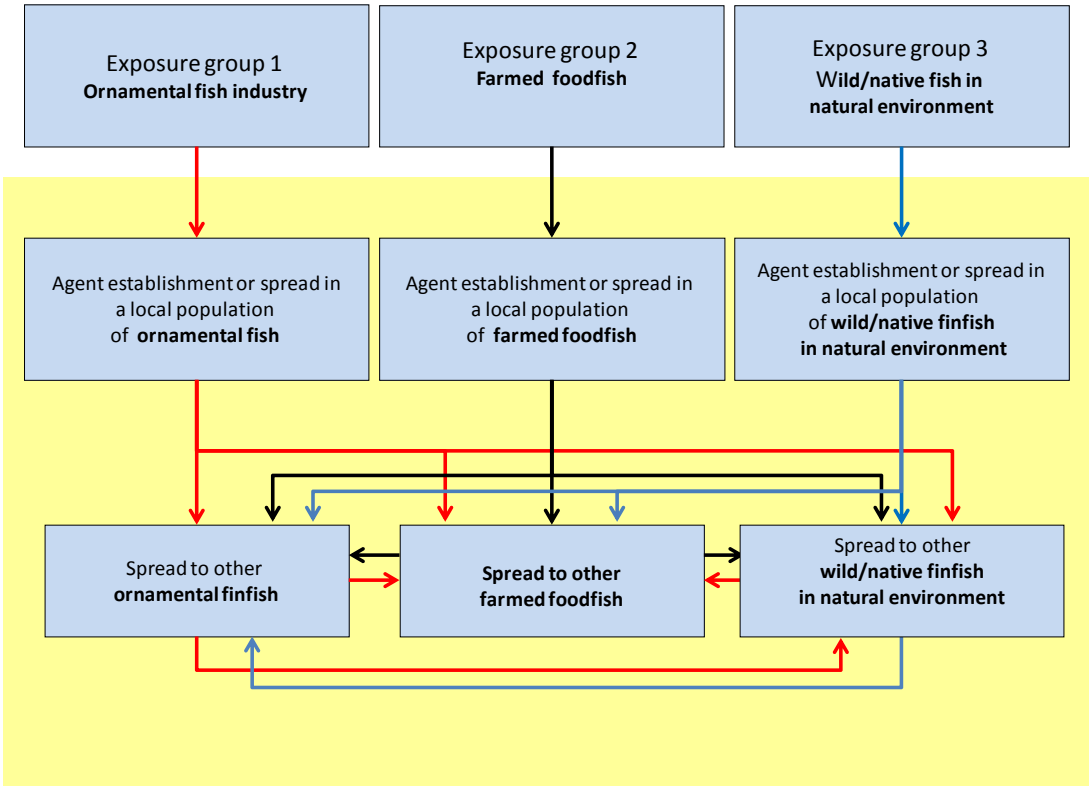
4. Combination of the likelihood of establishment or spread for that outbreak scenario with the corresponding estimation of adverse impacts to obtain an estimation of 'likely consequences' for each exposure group.

Identification of outbreak scenarios

Once exposure of susceptible host population has occurred, a number of possible outbreak scenarios could follow. Although these represent a continuum ranging from no spread to establishment of widespread endemic disease, for risk assessment purposes in this review, only one likely scenario has been assessed for each exposure group; namely, *the agent establishes or spreads in exposed populations and spreads further to other natural and farmed populations of susceptible host species in Australia*.

With respect to amphibian ranaviruses, susceptible host species may include amphibians. Amphibians that cohabitate with fish (for example, goldfish) in ponds may become infected with the virus or may act as a vector. For each iridovirus of quarantine concern, the likelihood of establishment or spread and the associated overall impact for the outbreak scenario was determined. In the identified outbreak scenario, it was assumed that if an agent were to establish or spread in a local population of susceptible host species through the various pathways shown in Figure 4, it would eventually spread to its natural geographical limits.

Figure 4 Establishment or spread pathways



Likelihood associated with outbreak scenarios

When estimating the likelihood of establishment or spread associated with the outbreak scenario, qualitative descriptors such as *negligible*, *low* and *moderate* are used as detailed previously (see Table 2, section 2.3).

Adverse (economic, biological and environmental) impacts of establishment or spread

The potential impacts of establishment or spread may be direct or indirect. Adverse impacts were evaluated in terms of seven (two direct and five indirect) impact criteria.

Direct impacts are those on:

- the life or health (including production effects) of production, domestic or feral animals. Note that impacts on ornamental and food fish aquaculture industries were considered under this direct criterion.
- the environment, including life and health of native wild animals and direct effects on the non-living environment. Note that aside from farmed salmonids and ornamental fish, all finfish species commercially farmed in Australia are native to Australia.

Indirect impacts are those on:

- new or modified eradication, control, surveillance or monitoring and compensation strategies or programs
- domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries
- international trade, including loss of export markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand
- indirect effects on the natural environment, including biodiversity, endangered species, and the integrity of ecosystems
- indirect effects on communities, including reduced tourism, reduced rural and regional economic viability, loss of social amenity, and any 'side effects' of control measures.

Consideration of the *indirect impacts on the environment* includes harm arising from the impact of the pathogenic agent itself, as well as from any treatments or procedures used to control it. The extent of harm was evaluated taking into account:

- all on-site and off-site impacts
- the geographical scope and magnitude of the impact
- the frequency and duration of the action causing the harm
- the total impact which can be attributed to that action over the entire geographic area affected, and over time (that is cumulative impact)

- reversibility of the impact; the sensitivity of the receiving environment (recognised environmental features of high sensitivity)
- the degree of confidence with which the impacts of the action are known and understood.

The direct and indirect impacts described collectively cover the economic, biological and environmental effects of a disease. In assessing direct and indirect impacts, it was important to ensure that particular impacts were not accounted for more than once. In particular, the direct impacts of a disease on native, non-commercial, wild populations were assessed under the criterion describing the 'environment, including the life or health of native animals', whereas the indirect or 'flow-on' effects on the environment were assessed under the last two indirect criteria.

Describing impacts

Estimating the overall impact associated with an outbreak scenario involved a two-step process where first, a qualitative descriptor of the impact of a pest or disease was assigned to each of the identified direct and indirect criteria in terms of the *level of impact* and the *magnitude of impact*. The second step involved combining the impacts for each of the seven criteria to obtain an overall impact estimation.

Step 1: Assessing direct and indirect impacts

Each direct and indirect impact was estimated at four levels—national, state or territory, district or regional, and local—and the values derived subsequently translated into a single qualitative score (A to G in Table 4). In this context, the terms 'national', 'state or territory', 'regional' and 'local', were defined as follows:

National	Australia-wide
State/territory	An Australian 'state' [New South Wales, Victoria, Queensland, Tasmania, South Australia or Western Australia) or 'territory' (the Australian Capital Territory, the Northern Territory, the Australian Antarctic Territory and other Australian Territories covered under the *Quarantine Act*)]. Note this excludes Christmas Island and the Cocos (Keeling) Islands.
District/region	A geographically or geopolitically associated collection of aggregates—generally a recognised section of a state or territory, such as the 'North West Slopes and Plains' or 'Far North Queensland'
Local	An aggregate of households or enterprises—for example, a rural community, a town or local government area

At each level, the magnitude of impact was described as 'unlikely to be discernible', of 'minor significance', 'significant' or 'highly significant':

- An 'unlikely to be discernible' impact is not usually distinguishable from normal day-to-day variation in the criterion.
- An impact of 'minor significance' is recognisable, but minor and reversible.
- A 'significant' impact is serious and substantive, but reversible and unlikely to disturb either economic viability or the intrinsic value of the criterion.

- A 'highly significant' impact is extremely serious and irreversible and likely to disturb either economic viability or the intrinsic value of the criterion.

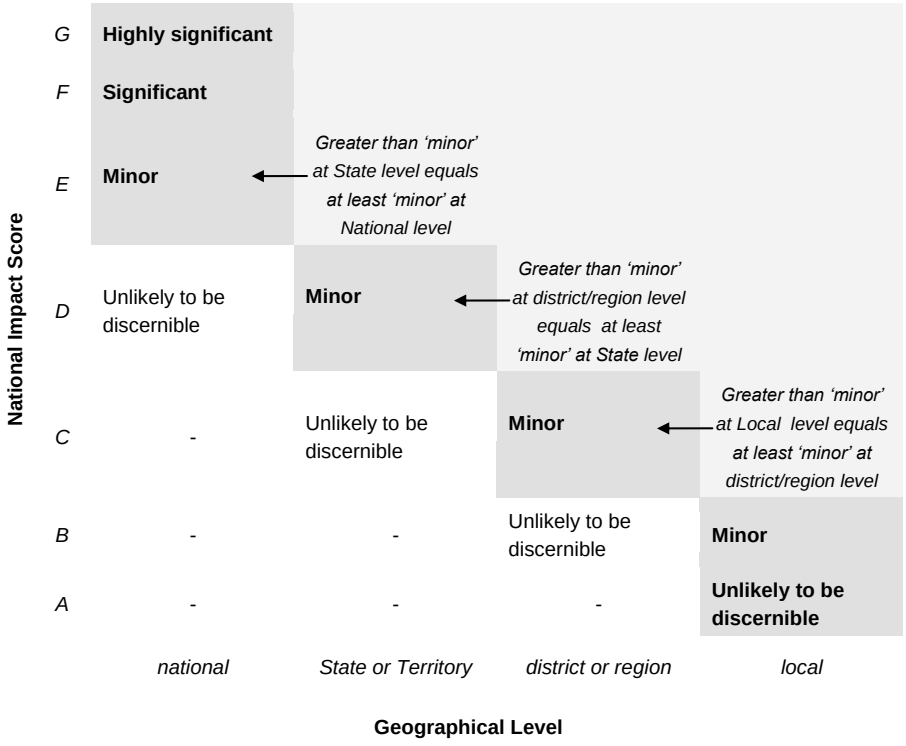
When assessing impacts, the frame of reference was the impact of each pathogenic agent on the community as a whole, rather than on the directly affected parties. A related consideration is the persistence of an effect. In general, the consequences were considered greater if the effect is prolonged, as would be the case if the agent was expected to persist for several production cycles or if restocking following eradication programs was expected to take several generations. If an effect is not prolonged, consequences are likely to be less serious.

Step 2: Combining direct and indirect impacts

To estimate the overall impacts of a disease outbreak on a national scale, it was necessary to combine the effects of the direct and indirect impacts on the national economy or the Australian community. The impacts were combined by first translating each individual direct or indirect impact to an overall score (A–G) using the schema outlined in Table 4. This was done by determining which of the shaded cells with bold font in the table corresponded to the level and magnitude of the particular impact. At each of the lower geographic levels, an impact more serious than 'minor' was understood to be discernible at the level above (for example, a 'significant' impact at the state/territory level would be considered to be equivalent to at least a 'minor' impact at national level). In addition, the impact of a disease at a given level in more than one state/territory, district/region or local area was considered to represent at least the same magnitude of impact at the next highest geographic level.

Once the appropriate shaded cell had been selected, the appropriate overall score for the outbreak scenario was assessed by reading the alphabetic (A–G) score from Table 4, starting at the national level and working down until the highest applicable combination of level and magnitude was reached. It is important to note that 'impact' at the national level is a different issue from 'spread of disease'. A disease may have serious consequences at the national level, despite only occurring in a small area.

Table 4 Assessment of direct or indirect impacts on a national scale[a]



a Shaded cells with bold font are those that dictate national impact scores. Impacts greater than 'minor' at local, district/regional or state/territory level are considered to represent at least 'minor' impacts at the next higher geographic level.

The measure of impact (A–G) obtained for each direct and indirect criterion was combined to give the overall impacts of a pathogenic agent. The following rules were used for the combination of direct and indirect impacts.

These rules are mutually exclusive, and should be addressed in the order that they appear in the list. For example, if the first set of conditions does not apply, the second set should be considered. If the second set does not apply, the third set should be considered, and so forth until one of the rules applies:

1. Where the impact of a disease with respect to any direct or indirect criterion is G, the overall impact is 'extreme'.
2. Where the impact of a disease with respect to more than one criterion is F, the overall impact is 'extreme'.
3. Where the impact of a disease with respect to a single criterion is F and the impact with respect to each remaining criterion is E, the overall impact is 'extreme'.
4. Where the impact of a disease with respect to a single criterion is F and the impact with respect to remaining criteria is not unanimously E, the overall impact is 'high'.
5. Where the impact of a disease with respect to all criteria is E, the overall impact is 'high'.

6. Where the impact of a disease with respect to one or more criteria is E, the overall impact is 'moderate'.
7. Where the impact of a disease with respect to all criteria is D, the overall impact is 'moderate'.
8. Where the impact of a disease with respect to one or more criteria is D, the overall impact is 'low'.
9. Where the impact of a disease with respect to all criteria is C, the overall impact is 'low'.
10. Where the impact of a disease with respect to one or more criteria is C, the overall impact is 'very low'.
11. Where the impact of a disease with respect to all criteria is B, the overall impact is 'very low'.
12. Where the impact of a disease with respect to one or more criteria is B, the overall impact is 'negligible'.
13. Where the impact of a disease with respect to all criteria is A, the overall impact is 'negligible'.

Combination of the likelihood of occurrence of each potential outbreak scenario with the estimated adverse impacts

The overall impact associated with the outbreak scenario was combined with the likelihood that the scenario would occur using the matrix in Table 5, so that a scenario-specific measure of 'likely consequences' was derived for the identified outbreak scenario per exposure group.

The result of the complete process was an estimate of the 'likely consequences' associated with the introduction of a pathogenic agent of concern into Australia.

Table 5 Matrix for estimating the 'likely consequences' for each outbreak scenario

Likelihood of establishment or spread	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
Very low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Extremely low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low
	Negligible	**Very low**	**Low**	**Moderate**	**High**	**Extreme**

Consequences of establishment or spread

2.4.5 Risk estimation

'Risk estimation' is the integration of 'likelihood of entry and exposure' and 'likely consequences of establishment or spread' to derive the risk associated with release, exposure and establishment or spread of a pathogenic agent of quarantine concern from the importation of freshwater ornamental fish for the identified exposure group.

As risks were associated with three exposure groups, risk estimation for this review was undertaken in two stages:

- estimation of the risk (of release, exposure and establishment or spread) for each of the three exposure groups
- combination of the risks associated with each exposure group to give an estimate of 'overall risk (of release, exposure and establishment or spread)'.

The risk associated with each exposure group was obtained by:

- determining the 'likelihood of entry and exposure' associated with each of the three exposure groups; and then
- combining the 'likelihood of entry and exposure' with the estimate of 'likely consequences of establishment or spread' obtained from the consequence assessment for each exposure group.

Combining the likelihood of entry and exposure and likely consequences of establishment or spread was undertaken using the 'rules' shown in the risk estimation matrix in Table 6.

Table 6 Risk estimation matrix

Likelihood of entry and exposure	Negligible impact	Very low impact	Low impact	Moderate impact	High impact	Extreme impact
High likelihood	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate likelihood	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low likelihood	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low likelihood	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

Consequences of entry and exposure

Estimation of overall risk

Risks of release, exposure and establishment or spread obtained for each of the three exposure groups were combined to give an overall estimate of risk. This was undertaken using 11 rules, which are mutually exclusive and should be addressed in the order that they appear in the list. For example, if the first set of conditions does not apply, the second set should be considered. If the second set does not apply, the third set should be considered, and so forth until one of the rules applies:

1. Where any one annual risk is extreme, the overall annual risk is also considered extreme.
2. Where more than one annual risk is high, the overall annual risk is considered extreme.
3. Where any one annual risk is high and each remaining annual risk is moderate, the overall annual risk is considered extreme.
4. Where a single annual risk is high and the remaining annual risks are not unanimously moderate, the overall annual risk is considered high.
5. Where all annual risks are moderate, the overall annual risk is considered high.
6. Where one or more annual risks are moderate, the overall annual risk is considered moderate.
7. Where all annual risks are low, the overall annual risk is considered moderate.
8. Where one or more annual risks are considered low, the overall annual risk is considered low.

9. Where all annual risks are very low, the overall annual risk is considered low.
10. Where one or more annual risks are very low, the overall annual risk is considered very low.
11. Where all annual risks are negligible, the overall annual risk is considered negligible.

The result of this process was an estimate of the 'overall risk of introducing an iridovirus of quarantine concern into Australia as a result of importing freshwater ornamental fish'. This was considered the final output of the risk assessment. Key steps in estimating the overall iridovirus associated risks are summarised in Figure 2 (section 2.4) and Table 7 (section 2.4.6).

2.4.6 Australia's appropriate level of protection

The SPS Agreement defines 'appropriate level of sanitary or phytosanitary protection' (ALOP) as the level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory.

Like many other countries, Australia expresses its ALOP in qualitative terms. Australia's ALOP, which reflects community expectations through government policy, is currently expressed as providing a high level of sanitary or phytosanitary protection aimed at reducing risk to a very low level, but not to zero. The band of cells in Table 6 (section 2.4.5) marked 'very low risk' represents Australia's ALOP.

Table 7 Estimation of overall annual risk

Likelihood/risk factor	Estimation/description
Release and exposure assessment	
Likelihood of *release*	Likelihood of *release*
Likelihood of *exposure* for each exposure group	Likelihood of *exposure*
Likelihood of *entry and exposure*	Estimated using the matrix of 'rules' for combining descriptive likelihoods (Table 3, section 2.3)
Consequences assessment	
Likelihood of *establishment or spread*	Likelihood of *establishment or spread* associated with the identified outbreak scenario
Overall impacts of *establishment or spread*	Outbreak scenario specific impacts (biological, economic and environmental) of *establishment or spread*
Likely consequences	Estimated by combining the likelihood of *establishment or spread* (associated with the outbreak scenario) with the estimated corresponding overall impact of *establishment or spread* for each exposure group using the matrix shown in Table 5 (section 2.4.4) to obtain likely consequences for each exposure group
Risk assessment	
The risk of *release, exposure and establishment or spread* associated with each exposure group	Estimated by combining the likelihood of *entry and exposure* with the likely consequences of *establishment or spread* for each exposure group using matrix shown in Table 6 (section 2.4.5) to obtain the risk of *release, exposure and establishment or spread* associated with each exposure group
Overall risk	Estimated by summing the risk of *release, exposure and establishment or spread* associated with each exposure group using the decision tool shown in section 2.4.4 to obtain an overall risk of *release, exposure and establishment or spread*

2.5 Risk management

Risk evaluation is described in the OIE as the process of comparing the estimated risk with a country's ALOP.

A risk that was either *very low* or *negligible* achieved Australia's ALOP. This provided a benchmark for evaluating risk and determining whether risk management is required.

The use of a benchmark for evaluating risk for each iridovirus is illustrated as follows:

- If the overall 'unrestricted risk' which in this IRA is based on existing risk management measures was negligible or very low, then it achieved Australia's ALOP and further risk management was not required.
- If the overall 'unrestricted risk' was low, moderate, high or extreme, risk management strategies were identified. These can be pre-export and post-arrival

measures (up to the point of release from quarantine) that are relevant to the 'likelihood of release' and/or post-arrival measures that are relevant to the 'likelihood of exposure' in the exposure scenario for each group of iridoviruses.

- The overall 'restricted risk' was then derived using a particular risk management measure or a combination of measures. If the 'restricted overall risk' is very low or negligible, that measure or combination of measures was considered acceptable.

2.5.1 Risk management measures

Risk management measures considered in this final IRA report are aimed at reducing the likelihood that the importation of freshwater ornamental fish from any country would lead to the *release, exposure and establishment or spread* of exotic pathogenic agents in Australia. There are two means by which this may be achieved:

- reducing the likelihood of pathogenic agents being released into Australia in imported freshwater ornamental fish by imposing risk management measures that reduce the likelihood of release, and
- reducing the likelihood that susceptible host species in Australia would be exposed to the pathogenic agent by imposing risk management measures that would reduce one or more of the likelihoods of exposure.

3 Ornamental fish industry

3.1 Ornamental fish industry in Australia

Over 1 billion ornamental fish are traded internationally each year (Whittington & Chong 2007), with 8–12 million imported into Australia alone (Department of Agriculture unpublished data).It is estimated that there are about 2000 species of ornamental fish in trade nationally and most of these are exotic to Australia (Moore et al. 2010). In 2006–7 the estimated domestic production was 8.3 million fish consisting of 7.7 million from aquaculture and 0.6 million from wild catch (O'Sullivan, Clark & Morison 2008).The ornamental fish trade in Australia has an estimated value of about $350 million annually (Natural Resource Management Ministerial Council 2006). This includes fish traded by commercial breeders, wholesalers, retailers and the hobby sector. The ornamental fish industry in Australia has a total annual retail turnover of $65 million (Natural Resource Management Ministerial Council 2006). In 2006–07 there were more than 200 licensed fish breeders, catchers and importers who supply to an estimated 1200 pet shops and specialist aquarium outlets around Australia through a network of wholesalers (O'Sullivan, Clark & Morison 2008). O'Sullivan, Clark and Morison (2008) also identified an active but difficult to quantify trade in fish within hobby associations and between enthusiasts. In addition, fish are also sold or swapped with retailers and wholesalers. An estimated 12 per cent to 14 per cent of the Australian population participate in the aquarist hobby at some level (Patrick 1998).

Aquaculture of ornamental fish in Australia occurs mainly in New South Wales, Victoria, Queensland and Western Australia (Love & Langenkamp 2003). Northern Territory production is negligible, with Tasmanian and South Australian production being mainly from single farms (J. Patrick, Pet Industry Association of Australia (PIAA), pers. comm. August 2005). Victoria is the largest producer of ornamental fish, both in terms of the number produced and dollar value, followed by Queensland, New South Wales and Western Australia (Table 8). Production values for Tasmania and South Australia were not available.

The Victorian ornamental fish industry is estimated to be worth tens of millions of dollars each year. Around 200 retail aquarium shops and a significant number of importers and breeders provide the public with a wide choice of fish and supplies.

Data on various ornamental fish types imported were available for one major importer in Victoria (averaged over the period from 2000–01 to 2004–05). Imports for this period consisted of poeciliids (57 per cent), goldfish (25 per cent), catfish (8 per cent), gouramis (8 per cent) and cichlids (2 per cent). Over the five years, the total number imported increased for all groups except catfish.

Of the total number of fish supplied to the Australian industry in the 1998–99 financial year, approximately 55 per cent of fish were imported and 45 per cent supplied by local breeders. In the 2002–03 financial year, approximately 59 per cent of fish were imported and 41per cent supplied by local breeders, although the PIAA reportedly estimates current domestic production to be as high as 60 per cent (Natural Resource Management Ministerial Council

2006). The details of the numbers of local ornamental fish production and its value are given in Table 8. Table 8 figures exclude hobby-level production (J. Patrick, PIAA, pers. comm. August 2005).The total number and value of imported ornamental fish (includes freshwater and marine ornamental fish) are provided in Table 8. The total number of fish imported grew from around 7.4 million in 1998–99 to over 14.4 million in 2010 but then dropped to about 11.8 million in 2012 (Department of Agriculture unpublished data). Of this 11.8 million, estimated 97.6 per cent are freshwater ornamental fish (estimated using the percentage of marine fish imported in 2012 [Department of Agriculture unpublished data]). Twenty-six countries are approved to export freshwater and marine ornamental fish into Australia, however only six countries account for 99 per cent of trade in this commodity. Department of Agriculture data on relative numbers of freshwater ornamental fish imported through Sydney (averaged over financial years 2001–02 to 2004–05) showed that goldfish made up 14 per cent of imports, cichlids 6 per cent, gouramis 4 per cent and others a total of 76 per cent. In 2012 the relative numbers of freshwater ornamental fish imported to Australia were very similar with goldfish at 16 per cent, cichlids 5 per cent, and gouramis 2 per cent and others 78 per cent (Department of Agriculture unpublished data).

Table 8 Total production[a] and value[b] of the domestic and imported ornamental fish trade

State	1998–99		1999–00		2000–01		2001–02		2002–03		2003–04		2004–05		2005–06	
	no. '000	$'000	no. '000	$'000	no. '000	$'000	no. '000	$'000	no. '000	$'000	no. '000	$'000	no. '000	$'000	no. '000	$'000
New South Wales	969.8	521.4	885.4	349.0	4784.3[c]	575.4	543.0	337.0	522.4	620.4	568.6	553.7	na[d]	547.0	na	429.0
Victoria	3543.0	2673.0	3587.0	2673.0	3569.0	2713.0	3871.0	3006.0	3875.0	3003.0	3957.5	2757.5	na	2741.0	NA	2523.0
Queensland	1506.9	676.0	1435.8	666.7	1546.8	823.0	2073.4	905.6	2686.8	986.9	1656.2	686.0	1868.7	920.0	2575.0	1230.6
Western Australia	na	na	126.5	168.0	288.0	288.0	300.0	421.0	124.6	331.0	114.7	326.0	na	336.0	na	483.0
Total domestic productione	6019.7	3870.4	6034.7	3856.7	5403.8	4399.4	6788.2	4669.6	7208.8	4941.3	6297.0	5523.2	na	4544.0	na	4665.6
Total import volumef	7483.0	2107.0	7400.0	2268.0	8151.0	2838.0	9053.0	3458.0	10648.3	3870.0	12545.7	4087.0	na	4749.0	na	5042.0

a Sources: (ABARE 2007; Department of Primary Industries 2004; How and Lawrence 2006; Lobegeiger and Wingfield 2007, 2006, 2005; Love and Langenkamp 2003; NSW Department of Primary Industries 2004; NSW Department of Primary Industries 2005). **b** Sources: (ABARE 2006; ABARE 2007; Australian Bureau of Statistics 2006). **c** This figure is incorrect and was to be revised by NSW DPI; however, to date this has not been done. **d na** not available. **e** 'Farm gate' values. **f** Freshwater and marine.

3.2 Regulatory control of ornamental fish production in Australia

State and territory legislation relating to fisheries and aquaculture, environment protection and land use planning, provides a key component of the legislative framework for aquaculture production, including ornamental fish (Productivity Commission 2004). Other state/territory, (such as, land administration, water management, conservation, native vegetation and food safety) and Australian government legislation (for example, environmental protection, native title and quarantine) may also affect aquaculture.

Under Australian government and state and territory legislation, various approvals for aquaculture production may be required, including leases, licences, permits and development approvals. Leases provide the right to occupy and use public land and waters for aquaculture purposes, and aquaculture licenses set out specific operating conditions. Other approvals such as development or planning approvals from local government and Australian government approval under the EPBC Act 1999 may also be needed. Table 9 provides a list of primary legislation governing fisheries or aquaculture in Australian states and territories.

Table 9 Primary legislation and supporting regulations governing fisheries or aquaculture in Australian states and territories

State or territory	Legislation governing aquaculture
Australian Capital Territory	*Fisheries Act 2000*
Northern Territory	*Fisheries Act 1988* Fisheries Regulations 1988
New South Wales	*Fisheries Management Act 1994* Fisheries Management (Aquaculture) Regulations 1995
Victoria	*Fisheries Act 1995* Fisheries Regulations 1998
South Australia	*Aquaculture Act 2001* Aquaculture Regulations 2005 *Fisheries Management Act 2007*
Western Australia	*Fish Resource Management Act 1994* Fisheries Resources Management Regulations 1995
Queensland	*Fisheries Act 1994* Fisheries Regulations 1995
Tasmania	*Inland Fisheries Act 1995*

All regulations pertaining to aquaculture apply to ornamental fish culture:

- In Tasmania, licensing of fish farms and registration of fish dealers are both relevant to the freshwater ornamental fish trade and fish cannot be imported, bred or sold without appropriate licence or registration.

- In South Australia, the *Aquaculture Act 2001* requires ornamental fish breeders to be licensed if their stock is used for the purposes of business, trade or research. Hobbyists that may engage in informal or more commercial trading of ornamental fish meet the definition of aquaculture under the *Aquaculture Act 2001*; however, few are licensed.
- In Queensland, anyone breeding or selling ornamental fish is required to hold a licence or a permit, although compliance/enforcement activities predominantly target large aquaculture ventures (including large ornamental fish breeders).
- In the Northern Territory, ornamental fish production is licensed under an aquaculture permit.
- In New South Wales, ornamental fish breeders are required to be licensed (Natural Resource Management Ministerial Council 2006).

Translocation policies generally control the translocation of exotic fish—fish that are not native (indigenous) to Australia—under aquaculture across state and territory borders. State and territory agencies can regulate exotic fish in the ornamental fish trade (usually) under the umbrella of fisheries regulation by declaration of noxious species (either via a prohibited list and/or a permitted list). They have the capacity to recall and seize fish species as required.

There are no specific controls on interstate movement of ornamental fish due to disease concerns other than for goldfish to Tasmania, which are aimed at managing risks associated with goldfish ulcer disease.

The Natural Resource Management Ministerial Council in its report, 'A strategic approach to the management of ornamental fish in Australia' (2006), recognised that ornamental fish present a significant risk to freshwater systems in Australia. Ornamental fish are recognised as having potential to trigger or contribute to future incursions of major aquatic pests or diseases. The strategy report identified the need for a nationally recognised noxious species (noxious fish are those that have been deemed harmful or produce conditions that are harmful to fisheries resources or habitat) list and harmonising the mechanisms or controls across regulatory agencies in Australia for the management and regulation of the ornamental fish trade. The report also recognises the importance of improved communication with all stakeholders and the wider community through communication plans. As part of this management strategy, a consistent regulatory framework for ornamental fish industry is planned. Effective implementation of such a framework may reduce the likelihood of disease spread from ornamental fish industry to farmed foodfish farms and fish in natural waters. The framework may also provide an avenue through which to monitor and control disease (including quarantine records to determine possible trends or to give advance warning of potential disease problems with offshore suppliers). Ornamental fish wholesalers and large ornamental fish breeders are a particular focus for state/territory regulatory control.

3.3 Industry codes of practice

In some states and territories, fish aquaculture industries have their own codes of practice that provide for self-imposed management of industry practices. However, codes of practice do not replace the need for aquaculturists to obtain and comply with all necessary licenses and approvals required by legislation. Aquaculturists include both foodfish hatcheries and grow-out operations and large ornamental fish producers who are involved in informal or more commercial trading of ornamental fish.

Queensland's aquaculture industry has an Industry Environmental Code of Best Practice for Freshwater Finfish Aquaculture that is endorsed by the state's Department of Agriculture, Fisheries and Forestry. The code includes water discharge control, escape prevention and disease management guidelines (Donovan 1999). Other state and territory fish aquaculture industries do not have environmental codes of practice that are endorsed by their respective state and territory authorities.

The ornamental fish retail sector does not fall specifically under fisheries regulation in most jurisdictions and would only be covered by a voluntary code of practice if retailers are members of the PIAA. However, only about 25 per cent of retailers are members of the PIAA (J Patrick, PIAA, pers. comm. January 2007).

The two largest ornamental fish wholesalers are members of the PIAA and account for about 75 per cent of ornamental fish imported into Australia (J Patrick, PIAA, pers. comm. January 2007). There are around 20 regular importers, about half of who are members of the PIAA (J Patrick, PIAA, pers. comm. January 2007). Department of Agriculture data (2012) show that there were 58 valid permits to import live ornamental fish. Of those, 37 are for freshwater and 21 are for marine ornamental fish.

PIAA's voluntary National Code of Practice, which includes a Code of Ethics, focuses on a number of issues including prevention of disease spread among aquatic animals, and reduction of the potential for establishment of non-indigenous species in the wild (see Appendix C). This code specifically addresses issues such as disposal of dead aquatic animals, and unwanted or sick fish. The PIAA code also encourages ornamental fish trade and industry practices in accordance with relevant state and territory regulations.

Although industry codes of practice for aquarium operations and aquaculture industries are voluntary, they can contribute to the management of risks posed by pathogenic agents.

3.4 Industry practices

In addition to information gathered from state and territory governments and PIAA, Department of Agriculture officers undertook industry site visits in Victoria and Queensland to gather information on industry practices relevant to this assessment. The department also held an industry workshop on 12 November 2008 aimed at adding to and verifying information gathered during site visits. The findings are summarised in sections 3.4.1 and 3.4.2.

3.4.1 The practice of feeding ornamental fish to farmed foodfish

Only Queensland has government controls or prohibitions on the use of live ornamental fish as food in freshwater fish farm operations, as it is an offence under the *Animal Care and Protection Act 2001* to feed a live fish to another fish. However, ornamental fish may be cohabitated together with foodfish broodstock in the same open ponds and under these conditions may be eaten by foodfish (B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005).

Feeding dead or live imported ornamental fish purchased from wholesalers and retailers to farmed foodfish broodstock is considered very rare because of the high cost of imported ornamental fish compared with low-cost, pond reared goldfish and poeciliids bred locally (B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005).

Some foodfish hatcheries may condition their broodstock by feeding live ornamental fish before commencement of the breeding season (B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005).

3.4.2 Contact between farmed ornamental fish and susceptible host species in natural waters

Australian breeders purchase imported fish as broodstock only when it becomes necessary to include new genetic broodstock in their breeding programs. For example, one major ornamental fish breeder has purchased new imported stock once in five years (B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005). Thus, it is likely that imported ornamental fish species are occasionally introduced into open ponds in farms. Escape of these fish from open ponds may expose fish in natural waters to imported ornamental fish and any pathogenic agents they might be carrying. The likelihood of that happening is very low due to biosecurity measures in place to reduce fish escaping in licensed commercial hatcheries. Further, most commercial breeders have in place disease prevention controls that include observation of newly introduced broodstock for a minimum of 30 days to reduce risks of introducing diseases.

Small-scale ornamental gourami pond aquaculture occurs in Australia, albeit to a lesser extent than that associated with goldfish and poeciliids. However, there was no evidence of ornamental cichlids being cultured in open ponds.

Goldfish are commonly reared in ponds in Australia and if not biosecure there is potential for amphibians cohabitating in the same pond to become exposed to pathogenic agents carried by ornamental fish.

3.5 Bait and berley survey

A 2002 survey of bait use in the Australian recreational fishing sector commissioned by the Department of Agriculture identified that ornamental fish may be used as bait in recreational fishing (assumed to include use in fishing for freshwater fish species)

(Kewagama Research 2002). The report indicated that freshwater fish species used as bait were either sourced from bait shops or were personally caught from the wild.

With respect to ornamental fish species, only the use (albeit it very rare) of guppies was specifically reported, with one report from Victoria and one from Queensland of guppies being sourced from bait suppliers, and two reports from Victoria of guppies being personally caught for use as bait. Respondents reported using both live and dead fish as bait (Kewagama Research, unpublished data). There was no indication that ornamental cichlids, goldfish or gouramis were used as recreational fishing bait.

Although both of the states visited (Queensland and Victoria) have legislation prohibiting the use of live fish as bait in freshwater, there was anecdotal evidence that some ornamental fish species such as goldfish, barbs and guppies were being used as bait for recreational fishing, both live and dead (B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005). Note that fishers may refer to gambusia (not considered an ornamental species) as 'guppies'.

3.6 Ornamental fish testing project

In 2005, the Department of Agriculture commissioned a project to determine the health status of imported cichlids, goldfish, gouramis and poeciliids while in post-arrival quarantine. These species were identified as high risk in the 1999 IRA. Diagnostic testing was conducted if a disease of quarantine concern was suspected, or when tank mortalities were increasing and exceeded 25 per cent within individual tanks. The trial therefore was not designed to detect the presence of pathogenic agents carried subclinically in imported ornamental fish. Diagnostic tests involved post mortem examination, histopathological and bacteriological examination, with provision for further confirmatory diagnosis as required.

One hundred cases were investigated from five mainland states of Australia. Viral aetiologies were diagnosed in seven submissions. Four cases of disease associated with iridovirus were diagnosed provisionally by histopathological examination (basophilic inclusions typical of megalocytiviruses) and two were confirmed by transmission electron microscopy. Note that if an exotic pathogen is reported during quarantine isolation, the consignment of imported ornamental fish is either destroyed or exported. The reported cases involved oscar (*Astronotus oscellatus*), rainbow krib (*Pelvicachromis pulcher*), and cichlids from the *Apistogramma* genus. All of these species belong to the family Cichlidae.

No cases of disease associated with iridovirus were diagnosed from goldfish, gouramis or poeciliids during the survey.

Although not found from gouramis sampled during this survey, DGIV of the genus *Megalocytivirus* has previously been reported in presumed imported gouramis in Australia (Go et al. 2006). Four cases of infection with DGIV – from imported ornamental fish submitted whilst in post-arrival quarantine – were also reported from Department of Agriculture submissions received during 2001–04 by the Western Australian Fisheries and Marine Research Laboratories before the survey. No cases of infection with iridovirus were reported from non-departmental submissions during the same period. These fish

populations in these submissions may contain locally bred ornamental fish as well as fish that were imported but have passed through quarantine.

Several cases of iridoviral infections have been reported from the Australian Department of Agriculture submissions (submitted during 2009 and 2010 only) received by the Western Australian Fisheries and Marine Research Laboratories since the ornamental fish testing project was completed in November 2006. No samples were submitted by the Department of Agriculture during 2007 and 2008. The reported cases involved cockatoo dwarf cichlid (*Apistogramma cacatuoides*), sky-blue dwarf gourami (*Colisa lalia*), dwarf gourami (*Colisa lalia*), lace pearl gourami (*Trichogaster leeri*), red wagtail platy (*Xiphophorus maculatus*), curviceps (*Laetacara curviceps*) and rainbow krib (F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009) (Department of Agriculture unpublished data).

4 Technical background

Iridoviruses are reported to cause disease and mortality in a wide variety of wild and farmed fish, including freshwater ornamental fish. Lymphocystis disease virus (LCDV) is endemic to Australia and is one of the most commonly reported iridoviruses. LCDV is known to cause cellular hypertrophy which produces elevated, multinodular skin tumours but rarely results in mortality (Wolf 1988). Epizootic haematopoietic necrosis (EHN), a systemic iridoviral disease caused by epizootic haematopoietic necrosis virus (EHNV), is listed by the World Organisation for Animal Health (OIE). EHNV causes high mortality in European or redfin perch (*Perca fluviatilis*) in the natural environment in Australia (Langdon 1986; Langdon and Humphrey 1987) but is not known to naturally infect any freshwater fish species on the Australian Government Department of the Environment *List of specimens taken to be suitable for live import*. EHNV is notifiable in all Australian States (South Australia, New South Wales, Western Australia, Tasmania, Victoria and Queensland) and the Northern Territory.

In research findings by the University of Sydney, Go et al. (2005) reported the detection of an iridovirus [dwarf gourami iridovirus (DGIV)] in ornamental gouramis in Australia and the experimental susceptibility (via cohabitation) of Murray cod (*Maccullochella peelii peelii*) to DGIV infection. These findings highlight both the potential for the introduction of exotic iridoviruses via imported ornamental fish and the potential susceptibility of Australian native fish. The spread of iridoviruses in other parts of the world has also been attributed to the importation and movement of infected live ornamental freshwater fish (Anderson et al. 1993; Grizzle and Brunner 2003; Hedrick and McDowell 1995; Mao et al. 1999). For example, iridovirus was isolated from healthy guppies (Poecilia reticulata) in the United States from South-East Asia (Grizzle and Brunner 2003; Hedrick and McDowell 1995). The isolated virus was later discovered to be closely related (Mao et al. 1999) but distinct from Santee-Cooper ranavirus (SCRV) (Holopainen et al. 2009).

Since Granoff et al. (1965) isolated an iridovirus from a leopard frog (*Rana pipiens* now known as *Lithobates pipiens*) with renal adenocarcinoma– the iridovirus was an incidental finding and not the cause of the tumour, amphibian infections with other iridoviruses or iridovirus-like viruses have been reported worldwide. A list of amphibian iridoviruses, their host range, and geographical distribution is provided in Table 10.

Iridoviruses are suspected of contributing to global amphibian population decline (Chinchar 2002; Daszak et al. 1999; Jancovich et al. 2001; Laurance et al. 1996). Some iridoviruses have been reported to infect animals from a range of animal taxa such as fish, amphibians and reptiles (Ariel and Owens 1997; Mao et al. 1997; Mao et al. 1999; Moody and Owens 1994). These viruses are increasingly being recognised as a serious threat to fish and amphibian populations, and as some appear to be capable of cross infecting animals in different taxonomic classes, it has been suggested that amphibians may be a reservoir for fish viruses and vice versa (Bayley and Hill 2007a).

4.1 Taxonomy of iridoviruses

Iridoviruses possess an icosahedral capsid, usually 120–200 nm in diameter, but up to 350 nm in the *Lymphocystivirus* genus (Chinchar et al. 2005). Iridoviruses contain a single molecule of double-stranded deoxyribonucleic acid (DNA) and some species acquire an envelope by budding through the host cell's membrane. The envelope increases the specific infectivity of the virions, but is not required for infection as naked particles are also infectious. Of note, iridovirus genomic regions are known to constantly change, providing potential for changes in pathogenicity to develop over time (Jeong et al. 2006).

The application of gene technology over recent years has enabled comparison and grouping of viruses at the molecular level that is helping to refine the taxonomy of iridoviruses. The family Iridoviridae comprises the genera *Iridovirus*, *Chloriridovirus*, *Lymphocystivirus*, *Ranavirus* and *Megalocytivirus* (previously referred to as cell hypertrophy viruses or tropiviruses) (Chinchar et al. 2005). The family also includes unassigned and uncharacterised iridoviruses (see Table 10). Members of the *Iridovirus* and *Chloriridovirus* genera are only known to infect invertebrate hosts, so are not discussed further in this risk analysis.

Lymphocystivirus genus

The genus *Lymphocystivirus* contains one recognised species, lymphocystis disease virus 1 (LCDV–1) and several species tentatively assigned to this genus, including lymphocystis disease virus 2 (LCDV–2). The disease has been observed in more than 100 fish species, although species of virus other than LCDV–1 or LCDV–2 may cause similar disease (Chinchar et al. 2005). Infection is characterised by gross hypertrophy of cells on the skin and fins of fish (Lewis and Leong 2004). Mortalities may occur under culture conditions, especially when infections involve the gills or when there is secondary bacterial infection (Chinchar et al. 2005).

Megalocytivirus genus

Due to the high degree of homology of the major capsid protein (MCP) gene sequences as well as similarity in the histopathological lesions induced, it has been accepted that iridoviruses causing severe systemic diseases and characteristic inclusion body bearing cells (IBC) in both freshwater and marine fish be classified into the *Megalocytivirus* genus (Chinchar et al. 2005).

Megalocytiviruses infect more than 30 species of cultured marine and freshwater fish belonging mainly to the orders Perciformes (perch-like fish) and Pleuronectiformes (flat fish) and are only known to infect teleosts (ray-finned fish) (Hyatt and Chinchar 2008).

Lee et al. (2009) reported swollen and degenerate cells with morphology consistent with leucocytes in the liver, spleen, kidney and other organs in rock bream (*Oplegnathus fasciatus*) infected with megalocytivirus. These leucocytes contained megalocytivirus DNA and virions, and the majority of such infected leucocytes were found within haemopoietic

tissues of the spleen, kidney and liver. The authors also showed that not all of these enlarged cells contain virus DNA.

Do et al. (2005a) compared the full MCP amino acid sequence of 13 flounder iridoviruses (FLIV) and an additional 31 iridoviruses available in GenBank and supported this classification. These authors also showed that there is considerable diversity of isolates within the genus.

The megalocytiviruses include DGIV, African lampeye iridovirus (ALIV) and infectious spleen and kidney necrosis virus (ISKNV). Go et al. (2006) compared the near complete MCP, adenosine triphosphatase (ATPase), ribonucleic acid (RNA) polymerase, IRB6 and CY15 gene segments of ISKNV, iridovirus isolates from gouramis (DGIV) presumed to have been imported and an iridovirus associated with mortalities in farmed Murray cod, known as Murray cod iridovirus (MCIV) in Victoria (Lancaster et al. 2003). They found these viruses to be minor variants of each other.

Jeong et al. (2008b) reported the detection of megalocytiviruses (ISKNV) in imported and locally bred pearl gouramis (*Trichogaster leeri*), silver gouramis (*Trichogaster trichopterus*) and dwarf gouramis (*Colisa lalia*) in South Korea. The authors reported natural infection of ISKNV in clinically infected (19 per cent prevalence) and asymptomatically infected fish (61 per cent) using 1-step polymerase chain reaction (PCR) and 2-step PCR, respectively. All 1-step PCR positive fish were gouramis, which showed mortality rates from 20 per cent –70 per cent and histopathological lesions typical of iridoviral infection. The authors also challenged each of ten pearl gouramis and silver gouramis with pearl gourami iridovirus (PGIV–l) by intramuscular injection, leading to 70 per cent and 20 per cent cumulative mortality within three weeks in pearl and silver gouramis, respectively.

In South Korea, Kim et al. (2010) detected megalocytivirus in dead and moribund paradise fish (*Macropodus opercularis*) imported from Indonesia. Of the 128 fish tested, 44 were shown to be PCR-positive for megalocytivirus (34 per cent).

Jeong et al. (2008b) also reported the detection of megalocytiviruses (ISKNV) in guppies (*Poecilia reticulata* = *Lebistes reticulatus*) (n=3), mollies (*Poecilia sphenops*) (n=4), platys (*Xiphophorus maculatus*) (n=6), swordtail (*Xiphophorus hellerii*) (n=3), oscars (*Astronotus ocellatus*) (n=2), neon tetras (*Paracheirodon innesi*) (n=2) and angelfish (*Pterophyllum eimekei* =*Pterophyllum scalare*) (n=7) using 2-step PCR, suggesting asymptomatic infection. These fish did not show clinical signs of infection or mortality and tested negative using 1-step PCR. This was the first report of megalocytivirus in neon tetras. As the sample size was very small (n=2), the agent was not retained as a hazard.

The megalocytiviruses also include a number of viruses reported from marine fish, such as red sea bream iridovirus (RSIV), sea bass iridovirus (SBIV), rock bream iridovirus (RBIV), rockfish iridovirus (RFIV), FLIV and grouper iridovirus (GIV) (Do et al. 2005a). Turbot iridovirus (TBIV) is also closely related to this group (Do et al. 2005a).

Do et al. (2005b) examined the phylogenetic relationship of strains of iridovirus within the *Megalocytivirus* genus and found that, based on the nucleotide sequence variation of the MCP gene, these viruses can be separated into distinct subgroups of viruses isolated from

marine fish (RSIV-like viruses) and those from freshwater fish (ISKNV-like viruses: ISKNV, DGIV and ALIV).

Song et al. (2008) examined the phylogenetic relationship based on the MCP gene nucleotide sequence and classified four virus isolates ISKNV (China), DGIV (Indonesia), MCIV and DGIV–4 (Australia) under one genotype. The nucleotide homology was greater than 99.5 per cent, suggesting that these viruses are of a single origin. Kim et al. (2010) classified the iridovirus detected in paradise fish under the same genotype.

Ranavirus genus

The *Ranavirus* genus contains many viruses that have been grouped into six species and which have been found to infect freshwater finfish, reptiles–snakes, lizards, turtles and tortoises and amphibians– frogs, toads, salamanders and newts (Chinchar et al. 2005; Hyatt and Chinchar 2008). Iridoviruses that infect amphibians, including frog virus 3 (FV–3), Bohle virus (BIV) and Ambystoma tigrinum virus (ATV), are also classified within this genus, as are finfish ranaviruses including EHNV, European catfish virus (ECV) and European sheatfish virus (ESV), Singapore grouper iridovirus (SGIV) (Chinchar et al. 2005), GIV (Tsai et al. 2005), short-finned eel ranavirus (Bovo et al. 1999) (SERV) also known as New Zealand eel virus (NZeelV). New Zealand eel is a freshwater fish but migrate to sea to breed and pike-perch iridovirus (PPIV) (Tapiovaara et al. 1998). Ranaviruses have been named according to the host from which they were first isolated, the type of disease or their place of origin.

Piscine ranaviruses

Epizootic haematopoietic necrosis virus

For the purposes of the OIE, EHN means infection with EHNV of the genus *Ranavirus* of the family Iridoviridae. However, the disease is caused by three closely related viruses: EHNV, ESV (Ahne et al. 1989; Ahne et al. 1990) and ECV (Pozet et al. 1992). EHNV, which is listed by the OIE, is endemic to Australia but ESV (isolated in Germany) and ECV (isolated in France and Italy) are exotic. ESV/ECV is currently listed on the OIE/Network of Aquaculture Centres in Asia-Pacific (NACA) regional list and is notifiable in South Australia and the Australian Capital Territory.

Challenge studies of redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) in Europe by Ariel and Bang Jensen (2009) have shown that European redfin perch and rainbow trout are not susceptible to natural infections with EHNV. However, Whittington and Reddacliff (1995) found populations of redfin perch in Australia to be highly susceptible to EHNV via bath challenge, even at very low virus concentrations.

Santee-Cooper ranavirus

There are several named isolates in the Santee-Cooper ranavirus (SCRV) species, including doctorfish virus (DFV), guppy virus (GV–6) and SCRV (Chinchar et al. 2005).

Pike-perch iridovirus

Pike-perch iridovirus (PPIV) was first isolated from apparently healthy pike-perch (*Stizostedion lucioperca*) fingerlings in Finland (Tapiovaara et al. 1998). This iridovirus is currently unassigned to any species under the *Ranavirus* genus. Holopainen et al. (2009) showed that PPIV is closely related to FV–3 based on restriction enzyme analysis (REA) of the DNA polymerase gene.

Short-finned eel iridovirus

Short-finned eel iridovirus was first isolated in Italy from asymptomatic short-finned eel (*Anguilla australis*) imported for human consumption from New Zealand in 1999 (Bovo et al. 1999). The virus is currently unassigned to a species under the *Ranavirus* genus.

Holopainen et al. (2009) showed that SERV was most closely related to ECV and ESV based on the MCP gene sequence, and closely positioned with ECV, EHNV, ESV and Ambystoma tigrinum virus (ATV) via amplification of the partial DNA polymerase gene. Amplification of the neurofilament triplet H1-like protein (NF-H1) gene showed that, phylogenetically, Bohle iridovirus (BIV), Rana tigrina ranavirus (RTRV) (also known as tiger frog virus—TFV) and frog iridovirus 3 (FV–3) form a cluster, PPIV and Rana esculenta virus (REV) group together and SERV lies apart from other isolates.

Amphibian ranaviruses

Amphibian iridoviruses are separated into three species, BIV, FV–3, and ATV (see Table 10) under the *Ranavirus* genus. Majji et al. (2006) have also provided evidence of a fourth possible species that infects amphibians (Rana catesbeiana virus RCV–Z).

Bohle iridovirus

While numerous amphibian iridoviruses have been described worldwide, only two, BIV (Speare and Smith 1992) and Wamena virus (WV) (Hyatt et al. 2002) have been identified in Australia. BIV, isolated from a native ornate burrowing frog (*Limnodynastes ornatus*) in northern Queensland (Speare and Smith 1992), was found to be highly pathogenic to barramundi (*Lates calcarifer*) following experimental infection using both inoculation and bath exposure (Moody and Owens 1994). BIV is considered endemic to Australia. Wamena virus (WV) isolated from illegally imported juvenile green pythons (*Chondropython viridis*) from Irian Jaya and may not be native to Australia.

Frog virus 3

Following the initial isolation of FV–3 from North America, numerous other FV3-like virus isolations have been reported from North America (Green et al. 2002; Greer et al. 2005; Mao et al. 1999; Miller et al. 2007), the United Kingdom (Cunningham et al. 1996), China (Zhang et al. 2006; Zhang et al. 2001), Thailand (Kanchanakhan 1998), Denmark (Ariel et al. 2009a) and South America (Fox et al. 2006; Galli et al. 2006; Zupanovic et al. 1998). Table 10 provides information on the various isolates of FV–3, the associated host species and the geographical distribution.

The FV3-like ranavirus (previously proposed name—Oxyeleotris marmoratus iridovirus (OMRV)) isolated in Thailand from diseased cultured marbled sleepy goby or sand goby (*Oxyeleotris marmoratus*) in 2000 (Prasankok et al. 2005) is now classified as RTRV—previously known as tiger frog virus under FV–3 species – together with other ranaviruses isolated from diseased cultured frogs (*Rana tigrina*) and goldfish (*Carassius auratus*) (Kanchanakhan et al. 2003).

Ambystoma tigrinum virus

Two ranavirus strains have been isolated independently from tiger salamander (*Ambystoma tigrinum*) epizootics in North America: ATV from Arizona, United States (Jancovich et al. 1997) and Regina ranavirus (RRV) from Saskatchewan, Canada (Bollinger et al. 1999). Isolating and characterising ranaviruses from tiger salamander epizootics in six states in the United States and two Canadian provinces in western North America have shown that these isolates had similar genomes, and are now recognized as a single widespread ranavirus species, ATV (Bollinger et al. 1999; Docherty et al. 2003; Jancovich et al. 1997). Salamanders are not found naturally in Australia.

Reptile ranaviruses

Ranaviruses have been isolated from an imported snake in Australia (Hyatt et al. 2002), turtles in China and the United States (Chen et al. 1999; De Voe et al. 2004), tortoises in Switzerland (Marschang et al. 1999), and a lizard in Germany (Marschang et al. 2005) (see Table 10). Whether the iridoviruses that infect reptiles can be genetically classified as ranaviruses is debatable as most studies have only sequenced a small portion of the MCP gene (Gray et al. 2009). However, an amphibian ranavirus isolate BIV was shown to be highly pathogenic to Australian native juvenile Krefft's tortoises (Emydura krefftii) and saw-shelled turtles (Elseya latisternum) when experimentally challenged (E. Ariel, James Cook University, pers. comm. January 2010). This study raises the possibility that reptiles may harbour amphibian ranaviruses but more research is needed to prove this hypothesis (Gray et al. 2009).

Unassigned viruses

Other unassigned iridoviruses include white sturgeon iridovirus (WSIV) and erythrocytic necrosis virus (ENV) (Chinchar et al. 2005), the former known from the United States and the latter widespread in marine fish.

Uncharacterised fish iridoviruses

Cichlid iridovirus (Armstrong and Ferguson 1989; Leibovitz and Riis 1980a) and angelfish iridovirus (Rodger et al. 1997; Schuh and Shirley 1990) cause lesions similar to those associated with megalocytiviruses with viral particles similar in size and shape being observed. Paperna et al. (2001) found similar iridovirus-like infections in moribund green swordtails (*Xiphophorus hellerii*), southern platyfish (*Xiphophorus maculatus*—commonly referred to as platys) and sailfin mollies (*Poecilia latipinna*). However, these viruses are yet to be characterised, although based on the presence of icosahedral particles within the

cytoplasm and characteristic enlarged cells (Chinchar et al. 2009) they are considered to be megalocytiviruses and thus are considered as such in this IRA.

Histopathological lesions similar to those associated with megalocytiviruses have also been demonstrated in cichlids and poeciliids in Australia from imported fish in quarantine.

Goldfish iridoviruses

Goldfish virus 1 and 2 (GFV–1, GFV–2) isolated in primary cell culture from healthy goldfish are considered to be distinct from lymphocystivirus and other iridoviruses causing systemic disease (Berry et al. 1983) and may constitute another genus (Lewis and Leong 2004). GFV–1 was isolated from healthy commercial stock of juvenile goldfish, while GFV–2 was isolated from a healthy wild adult goldfish. Both instances occurred in the United States and are the sole reported findings of GFV (Berry et al. 1983).

The hazard identification and refinement table (Table 1, section 2.1) in this document has been prepared by using information on iridoviruses associated with freshwater ornamental fish in Lewis and Leong (2004) and Chinchar et al. (2005), and incorporates other published information on megalocytiviruses and ranaviruses. Lewis and Leong (2004) provide a more extensive list of unassigned viruses than Chinchar et al. (2005) and have been grouped in a manner consistent with that of the International Committee on Taxonomy of Viruses (ICTV). Table 1 also includes amphibian ranaviruses relevant to the risk assessment that are listed in the database of the ICTV (Büchen-Osmond 2008).

Table 10 Host specificity of iridoviruses in fish, amphibians and reptiles

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
Megalocytivirus										
–	Infectious spleen and kidney necrosis virus									
–	–	African lampeye iridovirus (Japan imported from Singapore)	Norman's lampeye (*Aplocheilichthys normani*)	Norman's lampeye	na	Pearl Gourami	na	na	na	(Sudthongkong et al. 2002)
–	–	Dwarf gourami iridovirus (Australia from imported ornamental fish)	Dwarf gourami (*Colisa lalia*) Thick-lipped gourami (*Colisa labiosa*) Three-spot gourami (*Trichogaster trichopterus*) Pearl gourami (*Trichogaster leerii*) Murray cod (*Maccullochella peelii peelii*)	na	na	Murray cod	na	Murray cod	na	(Anderson et al. 1993; Go and Whittington 2006; Whittington et al. 2009)
–	–	Pearl gourami iridovirus (South Korea from imported and local ornamental fish)	Pearl gourami Silver gourami (*Trichogaster microlepis*) Dwarf Gourami	na	na	Pearl gourami and silver gourami[a] Rock bream (*Oplegnathus fasciatus*) Silver gourami	na	Rock bream	na	(Jeong et al. 2008b; Jeong et al. 2008a; Whittington et al. 2009)
–	–	Paradise fish iridovirus (South Korea from fish imported from Indonesia)	Paradise fish (*Macropodus opercularis*)	na	na	na	na	na	na	(Kim et al. 2010)

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
–	–	Infectious spleen and kidney necrosis virus (China)	Chinese perch (*Siniperca chuatsi*)	Chinese perch Grass carp (*Ctenopharyngodon idella*) (asymptomatic infection can be a carrier)	Nile tilapia (*Oreochromis niloticus*) Freshwater angelfish (*Pterophyllum scalare*) Mud carp (*Cirrhinus molitorella*) Mrigal (*Cirrhinus cirrhosus*) Crusian carp (*Carassius carassius*) Goldfish (*Carassius auratus*) Bighead carp (*Aristichthys nobilis*) Sliver carp (*Hypophthalmichthys molitrix*)	Chinese perch Largemouth bass (*Micropterus salmoides*) Zebrafish (*Danio rerio*)	Sea perch (*Lateolabrax japonicus*) Longspine grouper (*Epinephelus longispinis*) Yellow grouper (*Epinephelus awoara*) Brownstripe red snapper (*Lutjanus Vitta*) Gold spotted spinefoot (*Siganus punctatus*) Nile tilapia Freshwater angelfish Black porgy (*Acanthopagrus schlegelii schlegelii*) Red seabream (*Pagrus major*) Goldlined seabream (*Rhabdosargus sarba*) Snakehead (*Channa argus argus*) Barramundi (*Lates calcarifer*) Mud carp Mrigal	Chinese perch	Chinese perch	(He et al. 2002; Xu et al. 2008)

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
							Crusian carp Goldfish Bighead carp Sliver carp			
–	–	Iridovirus in mollies and platys (Australia[b] Israel and South Korea)	Southern platyfish (*Xiphophorus maculatus*) Sailfin mollies (*Poecilia latipinna*) Red wagtail molly (*Xiphophorus maculatus*) Molly (*Poecilia sphenops*)	na	na	na	na	na	na	(Jeong et al. 2008b; Paperna et al. 2001) (F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009)
–	–	Swordtail Iridovirus (Israel)	Green swordtail (*Xiphophorus hellerii*)	na	na	na	na	na	na	(Paperna et al. 2001)
–	–	Iridovirus in guppies (South Korea)	Guppy (*Poecilia reticulata*) (asymptomatic infection)	na	na	na	na	na	na	(Jeong et al. 2008b)
–	–	Cichlid Iridovirus (Canada imported from Singapore, South America, and the United States)	Orange chromide cichlid (*Etroplus maculatus*) Ram cichlid (*Mikrogeophagus ramirezi*) Nile tilapia (*Oreochromis niloticus niloticus*)	na	na	na	na	na	na	(Armstrong and Ferguson 1989; Leibovitz and Riis 1980a; McGrogan et al. 1998)
–	–	Angelfish iridovirus (United Kingdom and South Korea)	Freshwater angelfish	na	na	na	na	na	na	(Jeong et al. 2008b; Rodger et al. 1997; Schuh and Shirley 1990)

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
–	–	Iridovirus in oscars (Australia and South Korea)	Oscar (*Astronotus ocellatus*) (asymptomatic and clinically infected)	na	na	na	na	na	na	(Jeong et al. 2008b; Stephens et al. 2009; Whittington et al. 2009)
–	–	Iridovirus in Rainbow krib (Australia from imported fish	Rainbow crib (*Pelvicachromis pulcher*)	na	na	na	na	na	na	(Stephens et al. 2009)
–	–	Iridovirus in *Apistogramma* spp. – dwarf cichlids (Australia from imported fish)	*Apistogramma* spp.	na	na	na	na	na	na	(Stephens et al. 2009)
–	–	Iridovirus in curviceps (Australia from imported fish)	*Laetacara curviceps*	na	na	na	na	na	na	(F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009)
Ranavirus										
–	Epizootic haematopoietic necrosis virus									
–	–	Epizootic haematopoietic necrosis virus (Endemic in Australia)	Wild redfin perch (*Perca fluviatilis*) Farmed rainbow trout (*Oncorhynchus mykiss*)	Chinook salmon (*Oncorhynchus tshawytscha*) Mosquito fish (*Gambusia affinis*) Mountain galaxias (*Galaxias olidus*) Macquarie perch (*Macquaria*	Australian bass (*Macquaria novemaculeata*) Barramundi (*Lates calcarifer*) Australian smelt (*Retropinna semoni*) Tiger barb (*Puntius*	Australian bass Atlantic Salmon (*Salmo salar*) Redfin perch (Australia) Macquarie perch Golden perch Rainbow trout	Barramundi Goldfish Wild (European) Redfin perch at 20–22 °C)	Australian smelt (not infected) Mosquito fish Pike-perch European hatchery bred rainbow fish at 20 °C (No	Silver perch	(Ariel et al. 2009b; Ariel and Bang Jensen 2009; Bang Jensen et al. 2009; Bang Jensen 2009; Cinková et al. 2009; Gobbo et al. 2009; Gobbo

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
				australasica) Redfin perch (Australia) Murray cod (carrier) Hatchery bred European rainbow trout (20 °C[c]) Pike fry (*Esox lucius)* Zebrafish Angelfish Silver perch (*Bidyanus bidyanus*) Pearl gourami (*Trichogaster leeri)* Pike-perch (*Sander lucioperca*) Black bullhead (*Ameiurus melas*) at 15 °C and 25 °C Seabream (*Sparus aurata*) at 18 °C	*tetrazona*) Goldfish Sumatra barb (*Capoeta tetrazona*) Channel catfish (*Ictalurus punctatus*) Tilapia Wild European Redfin perch Rainbow trout (Australia) Golden perch (*Macquaria ambigua*) Koi carp (*Cyprinus carpio*) Goldfish Guppy	(Australia) Murray cod (carrier) Hatchery bred European rainbow trout (20 °C) Wild European redfin perch (15 °C and 20 °C) Silver perch Pike-perch		mortality but virus isolation only, suggesting a carrier status) Sunfish (*Lepomis cyanellus*)		et al. 2010; Hedrick and McDowell 1995; Langdon 1986; Langdon et al. 1988; Langdon 1989; Langdon and Humphrey 1987; Reschová et al. In press)
–	European Sheatfish virus									
–	–	European Sheatfish virus (Germany)	Farmed sheatfish (*Silurus glanis*)	Sheatfish Pike fry (12 °C) Pike-perch (possible carrier) Zebrafish (carriers at 20 °C and 28 °C) Guppy (carrier 20 °C, not susceptible at 28 °C) Angelfish (carrier at	Channel catfish (*Ictalurus punctatus*) Chinook salmon Koi carp Goldfish Redfin perch Rainbow trout	Pike-perch	–	Sheatfish	–	(Ahne et al. 1989; Ahne et al. 1990; Bang Jensen et al. 2009; Bang Jensen 2009; Cinková et al. 2009; Gobbo et al. 2009; Gobbo et al. 2010; Hedrick and McDowell 1995; Ogawa et al.

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
				20 °C not susceptible at 28 °C) Pearl gourami at 20 °C and 28 °C Black bullhead (15 °C)						1990)(S. Bergman Friedrich-Loffler Institute, pers. comm. January 2010)
	European catfish virus									
–	–	European catfish virus[d] (France and Italy)	Farmed European catfish *(Ictalurus melas)* Farmed turbot fry? Brown bullhead *(Ameiurus nebulosus)*	Pike-perch (possible carrier) Zebrafish (at 20 °C and 28 °C) Angelfish (20 °C) Pearl gourami at 20 °C and 28 °C Pike fry (subclinical carrier) Sheatfish Black bullhead (25 °C and15 °C)	Guppy Koi carp Goldfish Redfin perch Rainbow trout	Pike-perch European catfish[a]	–	European catfish	–	(Bang Jensen et al. 2009; Bang Jensen 2009; Bloch and Larsen 1993; Cinková et al. 2009; Gobbo et al. 2010; Jeremic et al. 2009; Pozet et al. 1992)
	Pike-perch iridovirus									
–	–	Pike-perch iridovirus (Finland from imported fish)	Farmed pike perch	Pike fry Pike perch (carrier at 12 °C and 22 °C)	Rainbow trout Redfin perch	–	Rainbow trout	–	–	(Bang Jensen et al. 2009; Bang Jensen et al. In press; Tapiovaara et al. 1998)
–	Short-finned eel iridovirus									
–	–	Short-finned eel iridovirus (Italy from NZ fish)	Farmed short-finned eel (*Anguilla australis)*	Pike fry Pike-perch (carrier at 12 °C and 22 °C)	Redfin perch Rainbow trout Black Bullhead (25 °C and 15 °C)	–	–	–	–	(Bang Jensen et al. 2009; Bang Jensen et al. In press; Bovo et al. 1999; Gobbo et al. 2010)
–	Santee-Cooper ranavirus									

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
–	–	Santee-Cooper ranavirus (United States)	Wild largemouth bass Florida bass (*Micropterus floridans*) Bluegill (*Lepomis macrochirus*) Striped bass Spotted bass (*Micropterus punctulatus*) Smallmouth bass (*Micropterus dolomieu*)	Largemouth bass Striped bass	–	Largemouth bass	–	na	Large-mouth bass[e]	(Goldberg 2002; Plumb et al. 1996; Plumb et al. 1999; Woodland et al. 2002; Zilberg et al. 2000)
–	–	Guppy virus/Doctorfish virus (United States imported from S/E Asia)	Farmed guppy (asymptomatic carrier) Farmed doctorfish (asymptomatic carrier)	Rainbow trout Chinook salmon Angelfish (Guppy virus only – carrier of DFV at 20 °C and susceptible at 28 °C) Zebrafish (carrier at 28 °C) Pearl gourami Pike (infection but no disease)	Redfin perch Channel catfish Goldfish Koi carp	na	na	na	na	(Bang Jensen 2009; Cinková et al. 2009; Hedrick and McDowell 1995; Reschová et al. In press)
–	Frog virus 3									
–	–	Frog virus 3[f] (United States)	Leopard frog (*Rana pipiens*)	Pike (carrier) Pike-perch (carrier) Larval stage of common frog (*Rana temporaria*) – 20 °C Larval stage common toad (*Bufo bufo*) – 20 °C Larval stage smooth	Guppy Koi carp goldfish Redfin perch Black Bullhead (25 °C and15 °C)	na	na	na	na	(Bang Jensen et al. 2009; Bayley and Hill 2007b; Cinková et al. 2009; Gobbo et al. 2010; Granoff et al. 1965)

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
				newt (*Triturus vulgaris*)						
–	–	FV3-like virus (United States)	Green frog (*Rana clamitans*) American bullfrog (*R. catesbeiana*)	na	na	na	na	na	na	(Gray et al. 2007; Miller et al. 2007)
–	–	FV3-like virus (Japan)	American bullfrog *Gnathopogon* spp.(family Cyprinidae)	na	na	na	na	na	na	(Une et al. 2009b)
–	–	FV3- like virus (Canada)	Wood frog (*Rana sylvatica*) Leopard frog	na	na	na	na	na	na	(Greer et al. 2005)
–	–	Tadpole edema virus (United States)	Bull frog (*Rana catesbeiana*)	na	na	Great basin spadefoot toad (*Scaphiopus hammondii intermontanus*) Young and adult American toad (*Bufo americanus*) Young and adult Fowlers toad (*Bufo Woodhousii fowleri*) Bullfrog	Rainbow trout Blue gill fry (*Lepomis macrochirus*) Salamanders	na	na	(Wolf et al. 1968)
–	–	Redwood park virus (United States)	Wild adult red-legged frog (*Rana aurora*)	na	na	na	na	na	na	(Mao et al. 1999)
–	–	Stickleback virus[g] (United States)	Wild three-spine stickleback (*Gasterostelus aculeatus*)	na	na	na	na	na	na	(Mao et al. 1999)
–	–	Tadpole virus 2 (United	Wild red-legged frog tadpole	na	na	na	na	na	na	–

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
		States)								
–	–	Rana tigrina iridovirus or tiger frog virus (China and Thailand)[h]	Goldfish Farmed marble goby (*Oxyeleotris marmoratus*) Farmed Tiger frog (*Rana tigrina rugulosa*)	na	Redfin perch Rainbow trout	na	na	na	na	(Bang Jensen 2009; Kanchanakhan et al. 2003; Weng et al. 2002)
–	–	Lucke' triturus virus 1 (United States)	Leopard frog	na	na	Red Newt (*Notophthalmus viridescens*)	na	na	na	(Clark et al. 1968)
–	–	Rana temporaria United Kingdom iridovirus (RUK) (United Kingdom)	Common frog (*Rana temporaria*)	Common frog	na	Common frog	na	na	na	(Cunningham et al. 1996; Cunningham et al. 2007a; Drury et al. 1995)
–	–	Bufo bufo United Kingdom virus (United Kingdom)	Common toad (*Bufo bufo*)	na	na	Common frog (I/P and S/C)	na	na	na	(Cunningham et al. 2007b)
–	–	*Bufo marinus* Venezuelan iridovirus (Gutapo virus?) (Venezuela)	Cane toad (*Bufo marinus*)	na	na	Giant tree frog) (*Litoria infrafrenata*) –S/C injection)	Spotted marsh frog (*Limnodynastes tasmaniensis*) Brown striped frog (*L. peronii*) Eastern banjo frog (*L. dumerilii*) Dainty green tree frog (*Litoria gracilenta*)	–	–	(Hyatt et al. 1998; Zupanovic et al. 1998)

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
–	–	FV3-like ranavirus from Maine spotted salamander (SsME) (United States)	Eastern spotted salamander (*Ambystoma maculatum*)	na	na	na	na	na	na	(Docherty et al. 2003)
–	–	Tortoise virus 5 (United States)	Central Asian turtle (*Testudo horsfieldi*)	na	na	na	na	na	na	(Mao et al. 1997)
–	–	Soft-shelled turtle iridovirus (China)	Farmed soft-shelled turtle (*Trionyx sinensis*)	Soft-shelled turtle	na	Soft-shelled turtle[a]	na	na	na	(Chen et al. 1999)
–	–	Box turtle virus 3 (United States)	Captive eastern box turtles (*Terrapene carolina carolina*)	na	na	na	na	na	na	(Allender et al. 2006; De Voe et al. 2004)
–	–	Iridovirus from Hermann's tortise (Switzerland)	Hermann's tortoise (*Testudo hermanni*)	na	na	na	na	na	na	(Marschang et al. 1999)
–	–	Ranavirus (Austria imported from Ethiopia)	Adult leopard tortoise (*Geochelone pardalis pardalis*)	na	na	na	na	na	na	(Benetka et al. 2007)
–	–	Ranavirus (United States)	Gopher tortise (*Gopherus polyphemus*)	na	na	na	na	na	na	(Westhouse et al. 1996)
–	–	Ranavirus from a gecko (Germany)	Gecko (*Uroplatus fimbriatus*)	na	na	na	na	na	na	(Marschang et al. 2005)
–	–	Wamena virus (WV) (Australia illegally imported from Irian Jaya)	Juvenile green pythons (*Chondropython viridis*)	na	na	na	na	na	na	(Hyatt et al. 2002)

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
	Ambystoma tigrinum virus									
–	–	Ambystoma tigrinum virus (United States)	Tiger salamander (*Ambystoma tigrinum*)	–	Sunfish Leopard frog American bullfrog Tiger salamanders	–	Mosquito fish Sunfish American bullfrog	–	Rainbow trout (no infection) Smelt (no infection) Mosquito-fish (no infection) Tiger salaman-ders	(Jancovich et al. 1997; Jancovich et al. 2001)
–	–	Regina ranavirus (RRV) (Canada)	Tiger salamander	na	na	na	na	na	na	(Bollinger et al. 1999)
–	–	RRV like ranavirus (United States)	Western tiger salamander (*Ambystoma tigrinum diaboli*) (Utah) *Ambystoma tigrinum melanostictum* (North Dakota)	na	na	na	na	na	na	(Docherty et al. 2003)
	Tentative species of amphibians									
–	–	Rana esculenta virus (Europe)	Green frog (*Rana esculenta*)	Larval stage of common frog – 20 °C Larval stage common toad – 20 °C Larval stage smooth newt – 20 °C	na	na	na	na	na	(Bayley and Hill 2007b; Fijan et al. 1991)
–	Unclassified amphibian iridoviruses									
–	–	Virus isolated from	*Leptodactylus* spp.	na	na	na	na	na	na	(Zupanovic et al.

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
		Leptodactylus spp. (Venezuela)								1998)
–	–	Rana grylio virus (China)	Farmed pig (ranid) frog (*Rana grylio*)	na	na	na	na	na	na	(Zhang et al. 2001)
–	–	Virus isolated from *Atelognathus patagonicus* (Argentina)	Leptodactylid frog (*Atelognathus patagonicus*)	na	na	na	na	na	na	(Fox et al. 2006)
–	–	Virus isolated from *Rana catesbeiana* (Brazil and Uruguay)	Farmed bullfrog (*Rana catesbeiana*)	na	na	na	na	na	na	(Galli et al. 2006)
–	–	Virus isolated from *Pelophylax kl. Esculentus* (PEV) (Denmark)	Wild edible frog (*Pelophylax esculentus*)[i]	na	na	na	na	na	na	(Ariel et al. 2009a)
–	–	Virus isolated from *Alytes obstetricans* tadpoles and *Mesotriton alpestris cyreni* juveniles (CMT) (Spain)	Midwife toad (*Alytes obstetricans*) Alpine newt (*Mesotriton alpestris cyreni*)	na	na	na	na	na	na	(Balseiro et al. 2009)
–	–	Ranavirus in wood frog (United States)	Wood frog (*Rana Sylvatica*)	na	na	na	na	na	na	(Harp and Petranka 2006)
–	–	Ranavirus from *Rana plancyi chosenica* (South Korea)	Gold-spotted pond frog (*Rana plancyi chosenica*)	na	na	na	na	na	na	(Kim et al. 2009)
–	–	Ranavirus	Not given	Tadpoles of	na	na	na	na	na	(Bayley et al.

Genus	Species	Isolate/strain	Susceptible hosts: natural infection	Susceptible hosts: experimental infection						References
				Bath immersion		Intraperitoneal injection		Cohabitation	Ingestion	
				Yes	No	Yes	No			
		(United Kingdom from imported live amphibians from the United States and Central America)		common frog (*Rana temporaria*) at 20 °C						2009)
–	–	Ranavirus (Japan)	Salamander (*Hynobius nebulosus*)	na	na	na	na	na	na	(Une et al. 2009a)
–	–	Virus isolated from Rana catesbeiana (RCV–Z) (United States)	American bullfrog	na	na	American bullfrog tadpoles	na	na	na	(Majji et al. 2006)

Bohle iridovirus										
–	–	Bohle iridovirus[j] (Australia)	Savannah water-holding frog (*Cyclorana brevipes*) Tadpoles and juveniles of green striped burrowing frog (*Litoria alboguttata*) Adult green tree frog (*Litoria caerulea*) Juvenile red-backed toadlet (*Pseudophryne coriacea*) Ornate burrowing frog (*Limnodynastes ornatus*)	Barramundi Juvenile green tree frog Zebrafish Pearl gourami Redfin perch Catfish (carrier)	Green tree frog (adults) Adult ornate nursery frog (*Cophixalus ornatus)* Angelfish Sharp snouted Torrent frog (*Taudactylus acutirostris*) Common toad Common frog Smooth newt Redfin perch Rainbow trout Koi carp Black Bullhead (25 °C and15 °C)	Green tree frog (juveniles) Desert tree frog (*Litoria rubella*) Bumpy rocket frog (*Litoria inermis*) Sharp snouted torrent frog (*Audactylus acutirostris*)	Green tree frog (adult) Juvenile cane toad Tilapia (I/M and I/P)	na	Tilapia	(Ariel and Owens 1997; Bang Jensen 2009; Bang Jensen et al. In press; Bayley and Hill 2007b; Cinková et al. 2009; Cullen and Owens 2002; Gobbo et al. 2009; Gobbo et al. 2010; Moody and Owens 1994; Speare and Smith 1992)

a By intramuscular injection. **b** Australian detections were from imported fish in post-arrival quarantine. The same applies to diagnoses in rainbow crib and *Apistogramma* spp. (Stephens et al. 2009). **c** Mortality significant at 20 °C only, suggesting it is not a natural condition for wild rainbow trout in Europe. **d** European isolates from sheatfish and catfish which were given distinct names are the same species, European catfish virus (Whittington et al. 2010). **e** Fed with dead guppies injected intraperitoneally with SCRV. Fish became infected but no clinical signs were observed. **f** Type species. **g** Same as Redwood creek virus. **h** No experimental transmission studies or epidemiological information available. **i** previously known as *Rana esculenta*. **j** Endemic to Australia.

4.2. Geographical distribution

Iridoviruses associated with freshwater fish are widespread in many parts of the world. Many epizootics are believed to have occurred in ornamental fish exports originating from various South-East Asian countries where these fish are held for short periods before export. Ranaviruses associated with freshwater fish have been reported in Australia (EHNV and BIV—only via experimental infection), Asia (GIV, SERV—from fish imported for human consumption to Italy from New Zealand, GV–6, RTRV and DFV), the United States (Stickleback virus and SCRV) and Europe (ESV, ECV and PPIV).

Megalocytiviruses

A number of cases of megalocytivirus infections have been reported in imported ornamental fish including Norman's lampeye (poeciliid) and a number of gourami species from South-East Asia, mainly Singapore (Anderson et al. 1993; Go et al. 2006; Paperna et al. 2001; Sudthongkong et al. 2002). The virus was also isolated from diseased three-spot gouramis (*Trichogaster trichopterus*) farmed in Florida, United States (Ahne et al. 1998; Fraser et al. 1993). Within megalocytiviruses, ISKNV in Chinese perch– formerly known as mandarin fish (Siniperca chuatsi) and an iridovirus infecting Murray cod were reported in China and Australia, respectively (He et al. 2000; Lancaster et al. 2003). Pearl gourami iridovirus has been reported in farmed and imported pearl gouramis in South Korea (Jeong et al. 2008b). Iridovirus has also been reported from South Korea in paradise fish (*Macropodus opercularis*) imported from Indonesia (Kim et al. (2010).

Paperna et al. (2001) reported systemic iridovirus infections in commercially reared gouramis and poeciliids in Israel that had been bred from fish imported from Singapore. Jeong et al. (2000b) also reported the detection of megalocytiviruses (ISKNV) in guppies, platys, swordtails and mollies using 2-step PCR suggesting asymptomatic infection from imported and farm reared fish in South Korea.

Iridoviruses have been associated with epizootic disease in farmed orange chromide cichlids (*Etroplus maculatus*) in Canada sourced from Singapore (Armstrong and Ferguson 1989) and ram cichlids (*Mikrogeophagus ramirezi*) in South America (Leibovitz and Riis 1980a). Iridovirus associated disease has also been reported from freshwater angelfish (*Pterophyllum scalare*) in the United Kingdom (Rodger et al. 1997). Jeong et al. (2008b) reported asymptomatic infection of oscars (*Astronotus ocellatus*) and angelfish in South Korea.

Iridovirus has also been detected via histopathological examination in a diseased oscar, a rainbow krib (*Pelvicachromis pulcher*) and a cichlid (*Apistogramma sp.*) in post-arrival quarantine in Australia during the ornamental fish testing project (Stephens et al. 2009). Since the completion of this project, further detections of iridovirus infections have been reported in fish in post-arrival quarantine; namely, cockatoo dwarf cichlid (*Apistogramma cacatuoides*), sky-blue dwarf gourami (*Colisa lalia*), dwarf gourami (*Colisa lalia*), lace pearl gourami (*Trichogaster leeri*), red wagtail platy (*Xiphophorus maculatus*), curviceps (*Laetacara curviceps*) and rainbow crib.

Ranaviruses

Piscine ranaviruses

An iridovirus infecting largemouth bass (*Micropterus salmoides*) was reported in south eastern United States (Plumb et al. 1999). Other iridoviruses within the SCRV group, infecting bluestreak cleaner wrasse, *Labroides dimidiatus* (named doctorfish in Hedrick and McDowell (1995)) and guppies imported from South-East Asia have also been reported from the United States (Hedrick and McDowell 1995). Restriction fragment length polymorphism (RFLP) generated after cleavage of radiolabelled viral DNA (with Hind 111 XbaI, BamHI and KpnI) has confirmed that SCRV (LMBV) is similar to DFV and GV–6 but markedly different from FV–3 (Chinchar and Mao 2000), although there is new information that GV–6 is more distinct from SCRV than previously considered (Holopainen et al. 2009). ECV and ESV have been reported from Europe.

Amphibian ranaviruses

The amphibian ranavirus RTRV (also known as tiger frog virus) has been isolated from goldfish in Thailand (Kanchanakhan et al. 2003). Rana temporaria (UK) ranavirus (RUK) associated with goldfish has been reported in the United Kingdom (Padgett-Flohr 2002) and although there was speculation of an epidemiological link between goldfish and frog deaths due to ranavirus, it has not been proven.

Japan reported an outbreak of FV3-like iridovirus in wild American bullfrog (*Rana catesbeiana*) larvae with high mortality (Une et al. 2009b). Cohabiting pond fish (of the families Cyprinidae and Gobidae) did not show clinical infection but liver tissues of fish *Gnathopogon* spp. (family Cyprinidae) were positive for ranavirus by PCR, suggesting asymptomatic infection.

Goldfish iridoviruses

GFV–1 was isolated from a primary cell culture derived from healthy commercial stock of juvenile goldfish, and GFV–2 was isolated from a primary cell culture derived from a wild adult goldfish, both in the United States (Berry et al. 1983).

4.3 Host range

Megalocytiviruses

Megalocytiviruses have been isolated from Chinese perch (He et al. 2000) and Murray cod (Lancaster et al. 2003), both belonging to the family Percichthyidae (temperate perch). Ornamental freshwater fish such as Norman's lampeye (Sudthongkong et al. 2002) and a number of gourami species (Anderson et al. 1993; Fraser et al. 1993; Go et al. 2006; Jeong et al. 2008b; Klinger et al. 1996; Paperna et al. 2001; Sudthongkong et al. 2002) have also been found to be infected with megalocytiviruses. Go et al. (2006) found dwarf gouramis (*Colisa lalia*), thick-lipped gouramis (*Colisa labiosa*), three-spot gouramis and pearl gouramis PCR positive for the MCP gene of DGIV. DGIV was successfully transmitted via cohabitation to Murray cod, resulting in clinical disease and mortality (Go et al. 2005; Go and Whittington 2006). Jeong et al. (2008b) reported megalocytiviruses in imported and local pearl gouramis, silver gouramis and dwarf gouramis in South Korea (see Table 10). Kim et al. (2010) reported megalocytivirus in paradise fish imported from Indonesia.

Iridoviruses, causing similar pathology to the megalocytiviruses have also been reported in ornamental poeciliids, including sailfin mollies, southern platyfish and green swordtails

(Paperna et al. 2001), and from several cichlid species (Armstrong and Ferguson 1989; Leibovitz and Riis 1980b; McGrogan et al. 1998; Rodger et al. 1997; Schuh and Shirley 1990). Jeong et al. (2008b) also reported the detection of megalocytiviruses (ISKNV) in oscars and angelfish using 2-step PCR suggesting asymptomatic infection. Iridovirus has also been detected in a diseased oscar, rainbow krib and a cichlid (*Apistogramma* sp.) in post-arrival quarantine in Australia during the ornamental fish testing project via histopathological examination (see Table 10). Further detections of iridoviral infections (via histopathology) have been reported in Australia from imported cichlids, poeciliids and gouramis whilst in quarantine (F. Stephens, Western Australian Fisheries and Marine Research Laboratories, pers. comm. October 2009). Natural infections with iridoviruses in the SCRV species have been reported in largemouth bass (Plumb et al. 1999), healthy bluestreak cleaner wrasse and healthy guppies (Hedrick and McDowell 1995).

Goldfish iridoviruses have only been isolated from primary cell culture derived from healthy goldfish (Berry et al. 1983).

Ranaviruses

The natural host range of ranaviruses in fish, amphibians and reptiles is shown in Table 10. The host specificity of ranaviruses is unknown. Experimental studies indicate that they are multi-host pathogens (Ariel and Owens 1997; Bang Jensen et al. 2009; Cullen and Owens 2002; Langdon 1989; Moody and Owens 1994; Schock et al. 2008). This is supported by the isolation of the same ranavirus in different amphibian species during a number of natural outbreaks (Duffus et al. 2008; Gray et al. 2007; Green et al. 2002).

Although inter-class infections have been documented following experimental infection with vertebrate iridoviruses, there are only two reports of the same virus being isolated from fish and amphibians in the same pond under 'natural' conditions (Mao et al. 1999; Une et al. 2009b). In another study, mosquito fish (*Gambusia affinis*) and sunfish (*Lepomis cyanellus*) did not become infected when exposed to ATV (Jancovich et al. 2001) suggesting that the type of ranavirus, the host species and the environment (for example, temperature) may determine whether fish can act as a reservoir of amphibian ranaviruses (Ariel and Bang Jensen 2009; Gray et al. 2009; Whittington and Reddacliff 1995).

4.4 Agent stability

A review of the scientific literature revealed little data on the stability of iridoviruses. Iridoviruses have been isolated from aquatic environments including from marine habitats, and are prone to inactivation by desiccation or heat at temperatures above 50 °C but are stable in water at 4 °C for extended periods (Williams et al. 2000). They are also known to retain infectivity through the typical laboratory isolation process, which includes freezing (at –30 °C) and thawing (Berry et al. 1983; Fraser et al. 1993).

Ranaviruses are capable of surviving within and outside their biological hosts. The resilient nature of the virus indicates a potential for spread via fomites, live fish, bait fish and skin surfaces of predatory animals such as birds (Hyatt and Chinchar 2008). EHNV is reported to survive more than 113 days on dry surfaces at 15 °C (Langdon 1989). The temperature range for replication of FV–3 is 15–30 °C (Zupanovic et al. 1998).

Information on iridovirus resistance to physical and chemical action is summarised in Table 11.

Table 11 Resistance of iridoviruses to physical and chemical action

Genus	Species	Isolate/strain	Treatment	Time	Infectivity
Megalocytivirus					
–	Infectious spleen and kidney necrosis virus				
–	–	Infectious spleen and kidney necrosis virus	–20 °C	>18 months	Remain infective
–	–		–70 °C		
–	–		50 °C	30 mins	Inactivated
–	–		40 °C	30 mins	Remain infective
–	–		30 °C	30 mins	
–	–		25 °C	15 days	
–	–		4 °C	6 months	
–	–		Sodium hypochlorite (200ppm at 25 °C)	15 mins	Inactivated
–	–		Formalin (concentration >2000ppm at 25 °C)	15 mins	
–	–		Potassium permanganate (concentration above 100ppm at 25 °C)	15 mins	
–	–		Ultraviolet radiation[a]	30 mins	
–	–		Iodine (100ppm at 25 °C)	15 mins	Remain infective
–	–	Dwarf gourami iridovirus	Frozen at –80 °C	na[b]	Remain infective
–	–	Pearl gourami Iridovirus	25 °C	4 days	Remain infective (more than 25% copy cells in controls)
Ranavirus					
–	Santee-Cooper ranavirus				
–	–	Santee-Cooper ranavirus	–10 °C	5 months	1– 6.8 $\log_{10}$ reduction
–	–		–16 °C	na	Remain infective (transmission studies in guppies)
–	–		Unknown temperature in water	2 days	Remain infective
–	–	Amphibian ranaviruses	Chlorhexidine (concentration 0.75%)	1 min	3 $\log_{10}$ reduction
–	–	–	Sodium hypochlorite (concentration 3.0%) Sodium hypochlorite (concentration 5.0%)	1 min 1 min	8 $\log_{10}$ 8 $\log_{10}$
–	–	–	Potassium permanganate (concentrations of 2 or 5 ppm)	60 mins	Not effective (only 1 $\log_{10}$ reduction)
–	–	–	Potassium peroxymonosulphate (concentration 1%)	1 mins 5 mins	Completely reduced at both time frames
–	–	–	Water, dried culture medium and frozen carcasses	na	Remain viable
–	–	–	4 °C ; –20 °C, – 7 °C	Prolonged periods	Remain viable
–	–	–	4 °C – 70 °C	One year	Remain infective
–	–	BIV	Desiccation at 42 °C	Six weeks	Remain infective
–	–	FV-3	50 °C	na	Lose infectivity

Genus	Species	Isolate/strain	Treatment	Time	Infectivity
–	–	–	pH 2–3	na	Lose infectivity
–	–		Irradiation	na	Inactivated
–	–	Tadpole oedema virus	56 °C	2 mins	Inactivated
–	–		pH 3 at 25 °C	30 mins	Inactivated
–	–	All iridoviruses	>55 °C	30 mins	Inactivated
–	–	Pike-perch iridovirus	50 °C	30 min	Inactivated
			pH 2.9	4 hours	
Goldfish iridoviruses					
–	–	Goldfish iridovirus 1 and 2 (viral suspension in media[c] or sterile distilled water)	56 °C	60 mins	Inactivated
–	–		pH 3	30 mins	
–	–		Chloroform	10 mins	
–	–		–30 °C	na	Remain infective

a UV lamp 20W, with wavelength 253.7 nm irradiated distance 50 mm for 30 mins. **b** na not available. **c** Hank's balanced salt solution (HBSS).

4.5 Epidemiology

Incubation period and carrier status

Megalocytiviruses

Go and Whittington (2006) reported 35 to 40 per cent cumulative mortality of Murray cod after 28 days of cohabitation with iridovirus infected gouramis in water at 27 °C. The donor gouramis were clinically healthy and were therefore carriers of the virus. Of the Murray cod that survived to 28 days, 32 per cent were shown to be PCR positive without any clinical signs, suggesting that it may take longer than 28 days for clinical signs of disease to develop. The same researchers reported 100 per cent mortality in Murray cod up to 21 days after injection with PCR positive gourami filtrates and demonstrated the potential for gouramis to harbour iridovirus without exhibiting clinical signs of disease for at least 28 days.

During disease outbreaks, the mortality of infected farmed Chinese perch reached 100 per cent within seven to eight days of the onset of clinical signs (He et al. 2000). Experimentally, juvenile Chinese perch injected intraperitoneally with ISKNV filtrates showed clinical signs six to ten days after infection at a water temperature of 28 °C and their mortality reached 100 per cent within 10 days of the onset of clinical signs (He et al. 2000). In two bath immersion trials, all Chinese perch immersed in water containing ISKNV filtrate died within 10–12 days after exposure. Animals showed similar clinical signs to the naturally affected fish (He et al. 2000; He et al. 2002). ISKNV has been detected from apparently healthy fish at temperatures below 20 °C (He et al. 2002).

Ranaviruses

Experimental transmission of EHNV to adult redfin perch using intraperitoneal and bath inoculation showed incubation periods of 11 days at a water temperature of 21 °C and 28 days at 12 °C (Whittington and Reddacliff 1995). In the same study, the disease was reproduced after intraperitoneal injection in juvenile rainbow trout at water temperatures ranging from 8–21 °C. The incubation period was three to ten days at 19–21 °C, but was up to 32 days at 8–10 °C. Ariel and Bang Jansen (2009) isolated EHNV virus in rainbow trout in the absence of significant mortality, suggesting a carrier role in the transmission of EHNV.

Guppy iridovirus has been found in healthy fish (Hedrick and McDowell 1995) suggesting that a carrier state is likely.

Ranavirus transmission studies suggest that ornamental fish including poeciliids, cichlids, gouramis and zebrafish (Danio rerio) can be carriers of ESV (Ariel 2009; Vesely et al. In press) and cichlids, gouramis and zebrafish can be potential carriers of ECV and GV–6 (Cinková et al. 2009). Plumb and Zilberg (1999b) found that largemouth bass developed clinical signs of disease three days after inoculation with SCRV.

Incubation periods associated with goldfish ranavirus and GV–6 are unknown.

Goldfish iridoviruses

GFV–1 and GFV–2 were isolated from healthy goldfish from primary cell culture of swim bladders and subsequent inoculation of CAR cells originally derived from goldfish fins (Berry et al. 1983). Cytopathic effect (CPE) was observed resulting in destruction of the monolayer within one week, with the isolate being confirmed by electron microscopy. Given that GFV–1 and GFV–2 were isolated from healthy goldfish, it is assumed that a carrier state exists.

Age susceptibility of the host

Megalocytiviruses

Juvenile to young adult dwarf gouramis infected with megalocytivirus (Anderson et al. 1993) and juvenile cichlids [freshwater angelfish, orange chromide cichlids and Nile tilapia (*Oreochromis niloticus niloticus*)] infected with cichlid iridovirus have been reported with clinical signs of disease (Armstrong and Ferguson 1989; McGrogan et al. 1998; Rodger et al. 1997). Murray cod infected with iridovirus in an outbreak in Victoria showed 90 per cent mortality in fingerlings 4–6 cm and 25 per cent mortality in fingerlings 10–15 cm over three to four weeks (Lancaster et al. 2003).

Ranaviruses

In amphibians, larval stages appear to be most susceptible to ranavirus infection but adults of some species are susceptible (Daszak et al. 1999). The age susceptibility of freshwater ornamental fish to ranaviruses is unknown, although Plumb et al. (1999) reported abnormal clinical signs from both adult and juvenile largemouth bass infected with SCRV suggesting adult and juvenile fish may both be susceptible. BIV was shown experimentally to be pathogenic to tilapia fry (via intraperitoneal and intramuscular injection) and barramundi fingerling (via bath immersion and ingestion) (Ariel and Owens 1997; Moody and Owens 1994).

Goldfish iridoviruses

No clinical signs of disease were observed in goldfish infected with GFV–1 and GFV–2 (Berry et al. 1983). GFV–1 was isolated from juvenile goldfish and GFV–2 from adult goldfish.

Host specificity

Megalocytiviruses

Megalocytiviruses are generally promiscuous, displaying a propensity to infect a wide range of host species. There are many experimental trials where virus from one host has been transmitted to another host to demonstrate lack of host specificity (see Table 10).

Ranaviruses

A survey of sympatric fish and amphibians collected from Redwood National Park in California showed that iridoviruses (Redwood Park virus) isolated from moribund three-spine stickleback (*Gasterostelus aculatus*) and a dead red legged frog (*Rana aurora*) tadpole were identical by restriction endonuclease profiles and sequence analysis of the MCP gene. These findings demonstrate that a specific ranavirus can naturally infect animals from different taxonomic classes and supports the hypothesis that amphibians serve as a reservoir for fish viruses and vice versa (Mao et al. 1999). However, while experimental infection trials by Bayley and Hill (Bayley and Hill 2007b) showed that some ranaviruses from amphibians are highly virulent to tadpole and post-metamorph life-stages of different amphibian species [such as the common frog (*Rana temporaria*) and common toad (*Bufo bufo*) to FV–3 and REV], there is no evidence that piscine ranaviruses are pathogenic to amphibian species.

Kanchanakhan et al. (2003) reported the isolation of RTRV from marble goby, goldfish and frogs. The Aquatic Animal Health Research Institute in Bangkok, Thailand, undertakes passive surveillance for RTRV on frog specimens submitted by farmers and fisheries officers. All RTRV isolations have been from clinically diseased specimens. However, despite continued surveillance, no ranavirus has been isolated from amphibians since 2007 (Kanchanakhan, Aquatic Animal Health Research Institute, Thailand, pers. comm. January 2010). In Asia, ranaviral infections have been reported from several cultured pig frogs (*Rana grylio*), tiger frogs (*Rana tigrina rugulosa*) and soft-shelled turtles (*Trionyx sinensis*) in southern China and from cultured tiger frogs in central Thailand (Chen et al. 1999; Weng et al. 2002; Zhang et al. 2001).

Although the route of introduction is unknown, scientists have speculated that an epidemic of ranaviral origin that has been killing Britain's most common amphibian species, the common frog (*Rana temporaria*), was linked to goldfish imported from the United States (Padgett-Flohr 2002). Although it is still speculated that ranaviruses may have originated from the United States, the route of transmission is still unknown (Cunningham et al. 2003).

To date, fish ranaviruses have not been associated with natural disease outbreaks in amphibians other than in the outbreaks in the Redwood Park in California and FV3-like ranavirus in Japan. Experimental studies have shown that virus from one host can be transmitted to another host to demonstrate lack of host specificity (Ariel and Owens 1997; Bang Jensen et al. 2009; Cullen and Owens 2002; Moody and Owens 1994) (see Table 10).

Goldfish iridoviruses

No information is available on the host specificity of GFV–1 and GFV–2.

Prevalence

Megalocytiviruses

Go et al. (2006) reported that 56 per cent of dwarf gouramis (n=18), 40 per cent of thick-lipped gouramis (n=5), 29 per cent of three-spot gouramis (n=35) and 8 per cent of pearl gouramis (n=39) tested positive for DGIV, using PCR analysis on samples of fish from two of the four Sydney pet shops sampled. Tissue homogenates from these infected fish were used as the source of virus to infect Murray cod in experimental transmission studies (Go and Whittington 2006). The prevalence of infection in Murray cod that survived 28 days after exposure to infected gouramis was 32 per cent (Go and Whittington 2006).

Jeong et al. (2008b) reported that 36 per cent pearl gouramis (n=36), 8 per cent silver gourami (n=13) and 25 per cent dwarf gouramis (n=12) tested positive for ISKNV viruses using 1-step PCR. In the same trial 56 per cent pearl gouramis, 77 per cent silver gouramis and 67 per cent of the dwarf gouramis were shown to be positive using 2-step PCR. Kim et al. (2010) reported that 34 per cent of dead and moribund paradise fish imported to South Korea from Indonesia tested PCR positive for megalocytivirus.

Ranaviruses

A survey using cell culture and PCR of 208 consignments of ornamental fish imported into the European Union from 14 countries did not reveal the presence of ranaviruses (Ariel 2009; Vesely et al. In press).

In Europe, from a total of 150 frogs tested from 30 consignments, ranavirus was isolated from frogs originating from the United States, Central America, Guyana, Ghana and Indonesia, suggesting the possibility of a worldwide distribution of ranavirus infection in the wild and possibly, captive amphibian populations. The American isolates were very closely genetically related to United Kingdom ranavirus isolates and FV–3 and so are likely to be strains of FV–3 (Hill and Bayley 2009).

Routes of transmission

Megalocytiviruses

Information on routes of natural transmission for megalocytiviruses associated with freshwater ornamental fish is limited; however, based on experimental transmission studies, horizontal transmission via cohabitation with infected fish, contaminated water, ingestion of infected excreta, or cannibalism of dead fish is likely.

Iridovirus in filtrate derived from tissue homogenates from presumed imported gouramis (from Sydney pet shops) was transmitted to juvenile Murray cod via intraperitoneal injection, causing 100 per cent mortality within 21 days (Go and Whittington 2006). The virus was also transmitted to Murray cod through cohabitation with infected gouramis (Go and Whittington 2006).

ALIV has been transmitted via immersion of Norman's lampeye in virus inoculated water and to pearl gouramis via intraperitoneal injection (Sudthongkong et al. 2002). ISKNV has been experimentally transmitted to juvenile Chinese perch via oral inoculation, bath immersion and cohabitation, as well as by intraperitoneal and intramuscular injection (He et al. 2000; He et al. 2002). The EHNV group of iridoviruses, including BIV, can be transmitted by intraperitoneal and intramuscular injection, via water or through cohabitation with infected fish (Langdon 1989; Moody and Owens 1994; Pozet et al. 1992; Reddacliff and Whittington 1996; Whittington and Reddacliff 1995).

Uninfected rock bream cohabitated with PGIV-challenged rock bream showed 100 per cent cumulative mortality indicating the potential for iridoviral transmission from freshwater ornamental fish to marine fish even in a marine environment (Jeong et al. 2008a).

Ranaviruses

Woodland et al.(2002) demonstrated oral transmission of SCRV to largemouth bass by feeding dead guppies spiked with virus (see Table 10).

Duffus et al.(2008) demonstrated that although vertical transmission is suspected, horizontal transmission of FV3 and FV3-like viruses through exposure to contaminated water is the most likely route of exposure in tadpoles.

In a study of the pathogenicity of ranaviruses in European freshwater fish species, pike (Esox lucius) fry were challenged via bath exposure with the ranaviruses EHNV, ESV, ECV, PPIV, SERV and FV–3 at 12 ° C and 22 °C (Bang Jensen et al. 2009). At 12 °C, significant mortalities were observed in fish exposed to EHNV, ESV, PPIV and SERV but infection with ESV and FV–3 showed no significant mortalities, although the virus could be isolated suggesting that pike may be a carrier. These findings suggest that pike fry are susceptible to EHNV, ESV, PPIV and SERV and can be subclinical carriers for ECV and FV–3.

Ariel and Bang Jenson (2009) showed that redfin perch and rainbow trout in Europe were not susceptible to EHNV to the extent reported previously in Australian studies. These results were confirmed in another study by Bang Jenson (2009) which also showed that European rainbow trout and redfin perch were not to be susceptible to ESV, ECV, PPIV, FV–3 and SERV. However, pike-perch were susceptible to EHNV and were potential carriers of ESV, ECV, PPIV and FV–3 at 12 °C, and ESV, ECV, PPIV, SERV and FV–3 at 22 °C.

In Europe, Reschová et al. (in press) studied the susceptibility of ornamental fish (zebrafish, guppies, angelfish, goldfish and carp) to a range of ranaviruses. The fish were challenged with EHNV, ESV, ECV, BIV, FV3, GV–6 and DFV via bath exposure at two temperatures (optimal temperatures ranges): zebrafish and angelfish at 20 °C and 28 °C, goldfish at 16 °C and 23 °C, and carp at 15 °C and 25 °C. The findings showed that zebrafish are susceptible to EHNV, ESV, ECV and BIV at 20 °C and to ESV, ECV, BIV, GV–6 and DFV at 28 °C. Guppies were susceptible to only ESV at 20 °C and although guppies are natural hosts for GV–6 (Chinchar 2002; Hedrick and McDowell 1995), they were not shown to be susceptible to GV–6 in this study. Angelfish were susceptible to EHNV, ESV, ECV, BIV, GV–6 and DFV at 20 °C. Carp and goldfish were not susceptible to ranaviruses in the study nor were they shown to be carriers. Only guppies were challenged with FV–3 and this group did not show significant mortalities.

Research into the susceptibility of pearl gouramis to ranaviruses showed that gouramis were susceptible to experimental infection with EHNV, ESV, ECV, BIV and GV-6 (Cinková et al. 2009).

Infectious dose

Megalocytiviruses

African lampeye iridovirus (ALIV) could be transmitted via immersion in a bath concentration of 10^5 tissue culture infectious $dose_{50}$ ($TCID_{50}$)/mL for two hours (Sudthongkong et al. 2002).

Ranaviruses

Infection trials with ranaviruses have used titres ranging from of 10^1–10^7 $TCID_{50}$ for all challenges (Ahne et al. 1990; Ariel and Bang Jensen 2009; Bang Jensen 2009; Hedrick and McDowell 1995; Langdon 1989; Tapiovaara et al. 1998; Whittington and Reddacliff 1995)..

Intraperitoneal injection of PPIV (Ranavirus group) at $5x10^4$ $TCID_{50}$ per dose caused infection, although clinical disease did not follow (Tapiovaara et al. 1998). In the same study, bath immersion at $2.5x10^3$ $TCID_{50}$/mL failed to establish infection.

Cohabitation with catfish injected intramuscularly with 10^3 plaque forming units (pfu) per dose achieved successful transmission of ECV (Pozet et al. 1992). Experimental bath inoculation with

as few as 0.08 $TCID_{50}$/mL of EHNV was lethal to redfin perch, but rainbow trout were resistant to bath exposure in $10^{2.2}$ $TCID_{50}$/mL and succumbed only after intraperitoneal infection (Whittington and Reddacliff 1995).

Intraperitoneal injection of a saline solution containing $10^{6.8}$ $TCID_{50}$ of LMBV infected more than 90 per cent of exposed fish (the titre of infected tissue was up to $10^{8.8}$ $TCID_{50}$/g), resulting in up to 80 per cent mortality at four days post infection (Plumb and Zilberg 1999b). BIV could infect barramundi fingerlings via intraperitoneal or intramuscular injection at 10^4 $TCID_{50}$ per dose or via bath immersion (freshwater) at 10^1 $TCID_{50}$ in a five litre tank (Moody and Owens 1994).

Tissue titres

Ranaviruses

LMBV concentrations of $10^{2.6\text{-}7.8}$ $TCID_{50}$/g of fish tissue have been reported in naturally infected largemouth bass (Plumb et al. 1999). Woodland et al. (2002) reported virus titres of $10^{2.8\text{-}9.5}$ $TCID_{50}$/g (the average weight of the fish was 64g) in largemouth bass orally infected with inocula of $10^{4.6}$~$10^{6.1}$ $TCID_{50}$.

Pozet et al. (1992) reported that ECV in fish injected with a dose of 10^3 pfu multiplied to at least 10^7 pfu/g of tissue. Tissue titres in fish infected via cohabitation with injected fish also reached 10^7 pfu/g.

4.6 Disease characteristics

Clinical signs

Megalocytiviruses

Cichlid iridovirus

The most commonly reported clinical signs associated with cichlid iridovirus infection are inappetence, generalised pallor (especially of the gills), respiratory distress, unusual swimming movements, abdominal distension, lethargy and exophthalmia (Armstrong and Ferguson 1989; Leibovitz and Riis 1980a; McGrogan et al. 1998; Rodger et al. 1997).

Infectious spleen and kidney necrosis virus species

Megalocytiviruses cause darkening of body colour and lethargy. Infected animals also exhibit severe anaemia, petechia of the gills and enlargement of the spleen.

He et al. (2002) reported depression, lethargy, unresponsiveness to disturbances, pale body pigmentation, cessation of feeding and gill pallor in ISKNV affected fish. Diseased Norman's lampeye, dwarf and pearl gouramis in both natural and artificial infections showed pale or a dark colouration of the body and sometimes ascites (Sudthongkong et al. 2002). Three-spot gouramis showed patches of hyper-pigmentation, lethargy and abdominal distension when infected with DGIV (Fraser et al. 1993). The typical clinical sign associated with iridoviruses found in gouramis was abdominal distension due to a pale enlarged kidney and spleen (Anderson et al. 1993; Fraser et al. 1993; Klinger et al. 1996).

Piscine ranaviruses

The clinical outcome of ranavirus infections varies from benign to fatal. Infections can lead to ulceration and/or systemic hematopoietic necrosis in amphibians and fish, and skin polyps, skin sloughing and systemic hematopoietic necrosis in salamanders.

Largemouth bass virus

An enlarged swim bladder causing abdominal distension is the only clinical sign of disease observed with natural infection (Plumb et al. 1999). However, Plumb and Zilberg (1999b) found that fish developed clinical signs three days after virus injection. The signs included dark pigmentation, spiral swimming behaviour, abdominal distension and lying listlessly on the bottom before death.

European catfish virus

Dead fish display classic clinical signs associated with infections by viruses with a tropism for haematopoietic tissues and the circulatory system. These include oedema, ascites and haemorrhages observable as petechiae around the pectoral and abdominal girdles and on viscera. Gill pallor is evident in many fish (Pozet et al. 1992).

European sheatfish virus

Apart from high mortality, clinical signs are non specific (Ahne et al. 1989). When experimentally infected, sheatfish showed anorexia, apathy and ataxia. Generally, moribund fish moved slowly with occasional sudden, rapid spiral movements.

Epizootic haematopoietic necrosis

Clinically moribund fish are dark in colour, inappetent and sometimes ataxic (Reddacliff and Whittington 1996).

Guppy virus–6

GV-6 has been isolated from apparently healthy fish and no clinical signs have been described.

Rana tigrina ranavirus

Diseased marbled goby exhibited minor ulcers on the body and around the mouth (Prasankok et al. 2005). Affected tiger frog tadpoles showed abdominal distension, ataxia and reduced feeding. No skin ulcers were observed in affected tadpoles (Weng et al. 2002).

Morbidity and mortality

Published information on mortality in fish infected with megalocytiviruses range from 50 per cent to 100 per cent (Table 12).

The single reported findings of infection with GFV–1 and GFV–2 were associated with healthy goldfish.

Table 12 Mortality associated with iridoviruses

Genus	Species	Isolate/strain	Host	Method of infection	Observation of clinical signs	Mortality	Reference
Megalocytivirus							
–	*Infectious spleen and kidney necrosis virus*						
–	–	African lampeye iridovirus	Norman's lampeye	Experimental (bath emersion)	na	100%	(Sudthongkong et al. 2002)
–	–		Pearl gourami	Experimental (intraperitoneal injection)		50%	–
–	–	Dwarf gourami iridovirus	Dwarf gourami	Natural	na	80%	(Anderson et al. 1993)
–	–		Gouramis (*Trichogaster spp*.)	Natural	24– 48 hours before death	<50%	(Klinger et al. 1996
–	–		Murray cod	Natural	4–7 days before death	90%	(Lancaster et al. 2003)
–	–			Experimental (intraperitoneal injection)	24 hours before death	>90%	(Go and Whittington 2006)
–	–			Experimental (cohabitation trials)	na	35–40%	
–	–		Three-spot gourami	Natural	24–96 hours before death	Up to 100%	(Fraser et al. 1993)
–	–	Pearl gourami iridovirus	Pearl gourami	Experimental (cohabitation trials)	na	60%	(Jeong et al. 2008a)
–	–	Pearl gourami iridovirus	Pearl gourami, dwarf gourami and silver gourami	Natural	na	20-60%	(Jeong et al. 2008b)
–	–		Pearl gourami	Experimental (intramuscular injection)	na	70%	
–	–		Silver gourami	Experimental (intramuscular injection)	na	20%	
–	–	Infectious spleen and kidney necrosis virus	Chinese perch	Natural	na	100%	(He et al. 2000)
–	–			Experimental (intraperitoneal injection and bath emersion)	6–10 days after infection	–	
–	–	Iridovirus in paradise fish	Paradise fish	Natural	na	100%	(Kim et al. 2010)
–	–	Angelfish iridovirus	Freshwater angelfish	na	na	>70%	(Rodger et al. 1997)
–	–	Cichlid iridovirus	Ram cichlids	Natural	–	40–80%	(Leibovitz and Riis 1980a)
–	–	Swordtail iridovirus	Green swordtail Mollies Platys	Natural	Yes	Yes. Mortality rates not reported	(Paperna et al. 2001)
Ranavirus							
–	Santee-Cooper ranavirus						
–	–	Guppy virus	Chinook salmon (*Oncorhynchus tshawytscha*)	Experimental (bath emersion)	na	5%	(Hedrick and McDowell 1995)
–	–		Rainbow trout	–	–	4%	–
–	–	Ranavirus (GV–6 DFV)	Pearl gourami	Experimental (bath emersion)	na	5–70% (20 °C) 25–58%	(Cinková et al. 2009)

Genus	Species	Isolate/strain	Host	Method of infection	Observation of clinical signs	Mortality	Reference
						(28 °C)	
–	–	Santee-Cooper ranavirus	Largemouth bass	Experimental (intraperitoneal)	3 days after infection	Up to 100%	(Plumb and Zilberg 1999b)
–	–			Experimental (bath emersion)	na	17%	
–	–			Experimental (intraperitoneal)		60%	(Plumb and Zilberg 1999b)
–	–		Striped bass (*Morone saxatilis*)	Experimental (intraperitoneal)		63%	(Plumb and Zilberg 1999b)
–	–			Experimental (bath emersion)		10%	
–	Frog virus 3						
–	–	RTRV	Goldfish	Natural	Yes but not described	na	(Kanchanakhan et al. 2003)
–	–	RPV	Stickleback	Natural	na	20%	(Mao et al. 1999)
	Epizootic haematopoietic necrosis virus						
–	–	EHNV	Redfin perch	Natural	Yes	High	(Langdon et al. 1988; Whittington et al. 1999)
–	–		Rainbow trout	Natural	na	0–0.1%	(Langdon et al. 1988; Whittington et al. 1999)
–	–		Macquarie perch	Experimental (bath emersion)	Yes	100%	(Langdon 1989)
–	–		Silver perch	Experimental (bath emersion)	Yes	30-67%	(Langdon 1989)
–	–		Mountain galaxias	Experimental (bath emersion)	Yes	100%	(Langdon 1989)
–	–		Pearl gourami	Experimental (bath emersion)	na	5–70% (20 °C) 25–58% (28 °C)	(Cinková et al. 2009)
–	–	ESV	Sheatfish	Natural Experimental (bath emersion	na Yes	90–100% 100%	(Ahne et al. 1989) (Ogawa et al. 1990)
–	–	–	Pearl gourami	Experimental (bath emersion)	na	5–70% (20 °C) 25–58% (28 °C)	(Cinková et al. 2009)
–	–	ECV	Catfish	Natural	na	90–100%	(Pozet et al. 1992)
–	–	–	Pearl gourami	Experimental (bath emersion)	na	5–70% (20 °C) 25–58% (28 °C)	(Cinková et al. 2009)
-	Bohle iridovirus						
–	–	BIV	Tilapia Barramundi	Experimental (ingestion) Experimental (intraperitoneal and Intramuscular)	na Yes	100% 100%	(Ariel and Owens 1997) (Moody and Owens 1994)
–	Tentative species of the genus ranavirus						
–	–	PPIV	Pike-perch	Natural	na	0%	(Tapiovaara et al. 1998)
–	–	SGIV	Singapore grouper	Natural	na	>90%	(Qin et al. 2003)

Genus	Species	Isolate/strain	Host	Method of infection	Observation of clinical signs	Mortality	Reference
Goldfish iridovirus							
–	–	Goldfish iridovirus 1 and 2	Goldfish	Natural	None	Mortality rate not reported (isolated in healthy goldfish)	(Berry et al. 1983)

na Not applicable.

Pathological signs

A summary of pathological signs associated with iridoviruses is provided in Table 13.

Table 13 Pathological signs and pathogenesis associated with iridoviruses

Genus	Species	Isolate/strain	Pathology and pathogenesis	Reference
Lymphocystivirus				
–	Lymphocystis disease virus 1			
–	–	Lymphocystis disease virus 1	Nodular hypertrophic growths, self-resolving lesions, stress-mediated.	(Smail and Munro 2001)
Megalocytivirus				
–	Infectious spleen and kidney necrosis virus			
–	–	All	Systemic disease with damage to the cells of the haematopoietic tissues in the kidney and spleen, formation of virus-infected hypertrophic cells disseminated in multiple tissues causing clinical anaemia.	(Smail and Munro 2001)
–	–	Cichlid iridovirus	Systemic disease in orange chromide cichlids with similar signs to megalocytiviruses infection (e.g. anaemia and hypertrophic cells being present). In ram cichlids, degenerative changes in the liver, spleen, kidneys, pancreas and eyes. Viral inclusion bodies in spleen.	(Smail and Munro 2001)
Piscine *Ranavirus*				
–	Epizootic haematopoietic necrosis virus			
–	–	Epizootic haematopoietic necrosis virus	Systemic disease with extensive necrosis of the haematopoietic tissues in the kidney and spleen, causing acute mortalities. Other organs are also affected including pancreas and the vascular endothelium within the liver, gill and heart.	(Chinchar et al. 2005; Langdon 1988; Langdon et al. 1988; Smail and Munro 2001)
–	–	European catfish virus	Destruction of kidney interstitial tissues and renal tubules and kidney and spleen haematopoietic tissues.	(Smail and Munro 2001)
–	–	European sheatfish virus	Generalized acute necrosis of splenic and renal haematopoietic tissue.	(Ogawa 1990)
–	Santee-Cooper ranavirus			
–	–	Santee-Cooper ranavirus	Systemic disease causing acute peritonitis, fibrinous exudate containing numerous leukocytes and abundant cellular debris in peritoneal cavity, necrosis of the liver, spleen, stomach and intestine.	(Zilberg et al. 2000)
–	–	Guppy virus	Asymptomatic in guppies. Haematopoietic and hepatocellular necrosis in the liver and kidney of experimentally infected rainbow trout and chinook salmon.	(Hedrick and McDowell 1995)
Amphibian *Ranavirus*				
–	–	Rana tigrina ranavirus	Hepatocellular necrosis throughout the liver and focal necrosis in the kidney in amphibians (tadpoles juveniles and adults).	(Weng et al. 2002)
Goldfish *Iridovirus*				
–	–	Goldfish iridovirus 1and 2	Isolated from swim bladder cultures of healthy goldfish. Subclinical carriers.	(Berry et al. 1983)

4.7 Diagnosis

Megalocytivirus

Iridovirus infection may be presumptively diagnosed by the observation of hypertrophied cells containing eosinophilic granular cytoplasmic inclusion bodies in the spleen or kidney (Schuh and Shirley 1990). Observation of CPE on cell culture may also be used, as some iridoviruses can be grown on a range of fish cell lines (Berry et al. 1983; Do et al. 2004; Fraser et al. 1993; Hedrick et al. 1992; Plumb et al. 1999; Pozet et al. 1992; Sudthongkong et al. 2002; Tapiovaara et al. 1998) although efforts to grow some of the megalocytiviruses infecting freshwater fish have failed (Anderson et al. 1993; He et al. 2002; Rodger et al. 1997). Ariel et al. (2009b) inoculated ten different ranavirus isolates on five different cell lines at five different temperatures and found that to detect all ranaviruses bluegill fry (BF-2), epithelio papilosum carpio (EPC) or chinook salmon embryo (CHSE-214) cell lines should be used at 20 °C, 24 °C or 28 °C.

Diagnosis may be confirmed by transmission electron microscopy (Armstrong and Ferguson 1989; Fraser et al. 1993; He et al. 2000; Hedrick et al. 1992; Paperna et al. 2001; Rodger et al. 1997; Schuh and Shirley 1990) or PCR analysis with primers mainly derived from virus DNA of the MCP or ATPase (Chao et al. 2004; Do et al. 2004; Do et al. 2005a; Go et al. 2006; Grizzle et al. 2003; Mao et al. 1999; Plumb et al. 1999; Sudthongkong et al. 2002).

Go et al. (2006) compared the near complete MCP, ATPase, RNA polymerase, IRB6 and CY15 gene segments of ISKNV, DGIV and MCIV. One of the PCR assays based on primer pair C50/C51 designed for the MCP gene was capable of detecting MCIV, RSIV and DGIV. This primer pair is also predicted to detect SBIV, giant sea perch iridovirus (GSIV), ALIV, RBIV and ISKNV and could be used as a screening test for megalocytiviruses (Go et al. 2006).

Go et al. (2006) also reported that a PCR assay with primer pair C82/C83 designed for the ATPase specifically from the MCIV sequence provided increased specificity for DGIV but was not able to detect RSIV. Neither assay recognised DNA from EHNV. Primers C50/C51 and C82/C83 could thus be applied in rapid diagnostic PCR tests for the presence of megalocytiviruses, differentiation of the DGIV-group from RSIV and exclusion of EHNV.

Whittington et al. (2009) reported the relative lack of sensitivity of detection of megalocytiviruses by genus-specific MCP primers C50/C51 from samples collected in 2004 (Go et al. 2006). This was addressed by redesigning within the same region of the MCP gene for genus-specific PCR of the *Megalocytivirus* genus using nested PCR. The MCP genes were checked against the latest sequence data in gene bank and the alignments showed that it would be likely that all megalocytiviruses for which sequence was available would be detectable using the primer pair C1105/C1106 and the use of the primer pair C1073/C1074 increased the sensitivity of the assay in a nested format (Whittington et al. 2009). The nested PCR detected both DGIV and RSIV groups.

Although DGIV-specific primer pair C82/83 has much greater sensitivity (especially on amplification), it also amplified RSIV control DNA, contrary to predictions based on sequence alignment. To overcome this problem, Whittington et al. (2009) used a new primer C1117 designed to replace C83, which did not amplify RSIV. The PCR assays developed for megalocytiviruses by Whittington et al. (2009) are shown in Table 14. Sensitivity and specificity of these tests are unknown.

Table 14 PCR assays developed for megalocytiviruses[a]

Target virus	Purpose	Gene	Primers	Type of assay
Megalocytivirus	Detect all known Megalocytivirus (both RSIV-like[b] and ISKNV-like[c] groups)	MCP	C1105/C1106 C1073/C1074	Nested PCR
Megalocytivirus	Detect ISKNV-like group only	ATPase	C1117/C82	Conventional or Real time PCR

a Based on Whittington et al. (2009). **b** RSIV-like = viruses from marine fish. **c** ISKNV-like = viruses from mandarin fish, gourami and Murray cod.

Ranaviruses

Most ranaviruses grow readily in cell culture using inoculates from both clinically infected and carrier fish (Langdon 1986).

Ariel et al. (2009b) found that the use of BG-2 cells at an incubation temperature of 22 oC, and an incubation period of two weeks followed by one week cultivation increases the likelihood of detecting ranaviruses in fish during surveillance, although EPC and CHSE-214 performed better in detecting all ranaviruses used in this trial; namely, FV3, BIV, PPIV, ECV, ESV, EHNV, DFV, GV–6, SERV and REV.

CPE in cell culture can be used to detect the presence of virus but does not identify the virus. Several techniques can be used to identify whether the virus is a ranavirus. Ranaviruses do not induce production of neutralising antibodies in the host and thus, all currently available methods are aimed at detecting virus antigens or ranavirus genes. These methods include enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT) (Hyatt et al. 1991). In instances where the virus does not grow in cell culture, histopathology followed by immunochemistry (from formalin fixed tissue) has been used (Reddacliff and Whittington 1996). However, these antigen detection methods based on polyclonal antibodies cannot be used to differentiate between ranaviruses because they target shared antigens.

Most viruses of the genus are very closely related in terms of the MCP gene, and can be differentiated by PCR in combination with restriction enzyme analysis (REA) or sequence analysis (Holopainen et al. 2009; Hyatt et al. 2000; Marsh et al. 2002; Une et al. 2009b).

In addition to differentiation of ranaviruses by MCP, Holopainen et al. (2009) used PCR and restriction enzyme analysis of DNA polymerase and neurafilament triplet H1-like (NF-H1) protein genes to differentiate ranaviruses.

5 Risk assessment

This chapter documents the assessment of risks, using the Department of Agriculture risk assessment framework, associated with importation of ornamental cichlids, goldfish, gouramis and poeciliids with respect to iridoviruses of quarantine concern. Note that reference to 'gouramis' in likelihood, impact and risk estimations that make up this risk assessment corresponds to fish of the subfamilies Luciocephalinae and Macropodinae of the family Osphronemidae.

Based on their phylogenetic relationships the iridoviruses of quarantine concern are grouped for the purposes of risk assessment as follows (see Table 15).

Table 15 Iridoviruses of quarantine concern retained for risk assessment

Iridovirus group	Fish group	Viruses considered
Megalocytiviruses[a]	Cichlids	Angelfish iridovirus, cichlid iridoviruses (chromide cichlid and ram cichlid iridoviruses), megalocytiviruses in oscars, rainbow krib, *Apistogramma* spp., and curviceps
	Gouramis	Dwarf gourami iridovirus, pearl gourami iridovirus, megalocytiviruses in silver gourami, thick-lipped gourami, three-spot gourami and paradise fish
	Poeciliids	African lampeye iridovirus, swordtail iridovirus, iridoviruses in mollies, platys, and guppies
Piscine ranaviruses	Poeciliids, cichlids, gouramis and zebrafish	Guppy virus 6, ESV/ECV
Amphibian ranavirus	Goldfish	RTRV
Goldfish iridoviruses	Goldfish	Goldfish iridovirus 1 and 2

a Although uncharacterised, in this review cichlid iridovirus, angelfish iridovirus and swordtail iridovirus are considered to be megalocytiviruses based on histopathology.

The likelihood and impact assessments made in this review are based on the information available and conclusions reached in the 1999 IRA, together with new information that has become available since that time. Thus, the likelihood estimations in this document take into account the quarantine measures currently in place for freshwater ornamental fish.

5.1 Release assessment

1999 IRA—key considerations

For iridoviruses of quarantine concern associated with freshwater ornamental fish, the 1999 IRA determined the likelihood of release to be low to moderate for cichlids and gouramis (Subfamily Luciocephalinae) and very low for other species.

In estimating the likelihood, in general terms, of an agent entering Australia through the importation of ornamental fish, the 1999 IRA based its estimations on the following criteria:

- host range and geographic distribution of the agent
- ease of agent detection
- expected prevalence in imported fish
- likelihood of agent presence in fish without causing overt disease.

In considering these criteria, the 1999 IRA took into account the following:

Industry factors

- The number of ornamental fish imported to Australia is decreasing, while the domestic ornamental fish breeding industry is expanding.
- An estimated 7 million freshwater ornamental fish were imported into Australia between 1996–7 (of these approximately 22 per cent comprised goldfish, 16 per cent poeciliids, 10 per cent cichlids, 6 per cent gouramis).

Source factors

- Healthy fish may carry pathogenic agents therefore appearing clinically normal at the time of export. The likelihood of shipments carrying subclinically infected fish would depend on the prevalence of disease in source populations.
- Prevalence of disease in source populations would vary between source populations, regions and countries.
- Standards of monitoring, surveillance and reporting would vary from one country to another, so that some regions or countries may appear to have a greater prevalence of disease than those countries where surveillance and reporting are given a lesser priority.
- Validity of available information on host range and geographical distribution depends on how easily the presence of the agent can be detected. Iridoviruses are particularly difficult to isolate and identify accurately, and require a relatively high degree of technical expertise.
- Fish that are obviously diseased are unlikely to be packed for export as poor quality shipments would lead to loss of trade.

Agent factors

The 1999 IRA also took into account the following factors in assessing the iridovirus-specific likelihood of agent entry into Australia:

- Freshwater ornamental fish iridoviruses in general have a wide geographical distribution, (at the time when the 1999 IRA was conducted) being reported from Singapore, South America, the United States and the United Kingdom.
- Iridovirus infection associated with high mortality and morbidity has been reported from cichlids and gouramis.
- Most infected fish are expected to develop clinical signs of disease 24–96 hours before death. Subclinically infected (apparently healthy) fish may be included in shipments exported to Australia.
- Activities at Department of Agriculture quarantine approved premises present a negligible likelihood of disease 'establishment' due to the required biosecurity procedures.

2010 IRA—new considerations

In addition to information presented in the 1999 IRA, the department considers the following information relevant to an estimation of the likelihood of release for each of the four iridoviruses of quarantine concern:

Industry factors

- The number of ornamental fish imported to Australia has increased. The total number of fish imported has grown significantly from around 7.4 million in 1998–09 to over 12.5 million in 2003–04. Of this, only 2 per cent are estimated to be marine ornamentals (estimated using the percentage of marine ornamental fish imported in 2006, Department of Agriculture unpublished data). The number of freshwater and marine ornamental fish imported in 2006 was estimated to be 15.8 million (AQIS 2006). Department of Agriculture data show that in 2008 approximately 19 million ornamental fish were imported to Australia.
- Groups of host fish species associated with iridoviruses of quarantine concern, namely cichlids, goldfish, gouramis and poeciliids, are still traded widely in the international ornamental fish market and represent significant numbers of fish imported annually into Australia.
- Based on data from one major wholesale importer, of the estimated 12.5 million freshwater ornamental fish imported into Australia in 2003–04 approximately 57 per cent comprised poeciliids, 25 per cent goldfish, 8 per cent catfish, 8 per cent gouramis and 2 per cent cichlids. Approximate percentages of ornamental fish species imported are based on data from one major wholesale importer. Although similar departmental figures are available, the numbers are reported only for cichlids, goldfish and gouramis. The other species including poeciliids and zebrafish are classified as one group thus making the percentages of imported poeciliids and zebrafish unknown.

Source factors

- Based on the 1999 IRA, modified pre-export and post-arrival quarantine conditions were introduced in 1999. The pre-export requirements include health certification by the competent authority of the exporting country attesting that:
  - Fish in the consignment have been inspected within seven days before export and showed no clinical signs of pests or infectious diseases.
  - Export facilities are currently approved for export to Australia as meeting standards set by the Department of Agriculture.
  - All fish held at export facilities exhibit no signs of significant infectious disease or pests and are sourced from populations not associated with any significant disease or pests within the six months before certification.
  - All fish in the consignment have been in facilities approved for export of freshwater fish to Australia for the 14 days before export.
- The 1999 import conditions require that the Department of Agriculture inspects all consignments of imported ornamental fish, ensuring correct documentation, including health certification issued by overseas competent authorities. Shipments of fish that are

not accompanied by correct documentation or that contain diseased animals or extraneous material or animals are destroyed or exported. On successful completion of post-arrival inspection, fish are ordered into quarantine approved places for minimum periods of between one to three weeks, depending on species (goldfish three weeks; cichlids and gouramis two weeks; all other species one week). The minimum 14-day post-arrival quarantine detention period for cichlids and gouramis was designed specifically to address the iridovirus associated risk. The department inspects fish and imposes controls on the procedures carried out at post-arrival quarantine approved places, including disposal of dead fish, wastewater, transport water and potentially contaminated packaging materials, and record keeping.

Go et al. (2006) and Go and Whittington (2006) isolated iridovirus from various gourami species collected from two Sydney pet shops. These fish may have been imported and if they were, then they would have been subjected to the current import requirements, including a 14-day period of pre-export observation in approved export facilities and 14 days post-arrival quarantine.

Agent factors

Megalocytiviruses

Go and Whittington (2006) demonstrated the potential for gouramis to harbour iridovirus without exhibiting clinical signs of disease for at least 28 days. These fish had already been kept in retail facilities where infection may have been acquired, but it is also possible that they carried the virus throughout the pre-export and post-arrival quarantine periods, suggesting a potential long-term carrier status. It is reasonable to assume that susceptible ornamental fish species may also harbour other iridoviruses of quarantine concern without showing clinical signs of disease. As such, healthy subclinically infected fish may not be detected during inspection at arrival or during pre-export and post-arrival quarantine.

Go et al. (2006) reported that 56 per cent of dwarf gouramis (*Colisa lalia*) (n=18), 40 per cent of thick-lipped gouramis (*Colisa labiosa*) (n=5), 29 per cent of three-spot gouramis (*Trichogaster trichopterus*) (n=35) and 8 per cent of pearl gouramis (*Trichogaster leeri*) (n=39) tested positive for DGIV, using PCR analysis on samples of fish from two Sydney pet shops. Murray cod (*Maccullochella peelii peelii*) were successfully infected with virus sourced from ornamental fish taken from a pet shop population that had previously tested negative for DGIV (Go and Whittington 2006).

Jeong et al. (2008b) reported that 36 per cent of pearl gouramis (n=36), 8 per cent of silver gourami (*Trichogaster microlepis*) (n=13) and 25 per cent of dwarf gouramis (n=12) tested positive for ISKNV viruses using 1-step PCR. In the same trial, 56 per cent of pearl gouramis, 77 per cent of silver gouramis and 67 per cent of the dwarf gouramis were shown to be positive on 2-step PCR. Some gouramis were shown to be 2-step PCR-positive without showing clinical signs, suggesting the existence of a carrier state.

Kim et al. (2010) reported that 34 per cent of paradise fish tested (n=128) were PCR-positive for megalocytivirus.

Jeong et al. (2008b) also reported that 83 per cent of platys (*Xiphophorus maculatus*) (n= 6), 100 per cent of mollies (*Poecilia sphenops*) (n=4), 67 per cent of guppies (*Poecilia reticulata*) (n=3), and 33 per cent of swordtails (*Xiphophorus hellerii*) (n=3) were positive for megalocytiviruses using 2-step PCR. These fish did not show clinical signs of infection or mortality during the

three-week holding period, suggesting a carrier state. In the same study, 14 per cent of angelfish (*Pterophyllum eimekei*) (n=7) and 100 per cent of oscars (*Astronotus ocellatus*) (n=2) were found to be positive on 2-step PCR.

A systemic iridovirus-like infection in a range of commercially reared poeciliids was reported in Israel (Paperna et al. 2001). Although currently uncharacterised, these viruses are considered to be megalocytiviruses based on histopathology (Chinchar et al. 2009).

Megalocytivirus infection has been reported in Japan in Norman's lampeye (*Aplocheilichthys normani*) imported from Singapore (Sudthongkong et al. 2002).

The ornamental fish testing project (2006) investigated more than 100 cases of imported fish from five mainland states of Australia. Investigations were not limited to suspect iridovirus cases but included any disease condition that caused mortality over 25% in the imported ornamental fish. Cichlids, goldfish, gouramis and poeciliids were targeted for diagnostic testing in the program if clinical disease was observed and an exotic pathogen was suspected. The diagnostic tests involved post mortem, histological and bacteriological examination with provision for further confirmatory diagnosis as required. Although four cases were positive for cichlid iridoviruses, no iridovirus infections were diagnosed from goldfish, gouramis or poeciliids during the survey period. However, four gourami iridovirus cases were found to be positive from departmental submissions received by the Western Australian Fisheries and Marine Research Laboratories prior to the survey (2001–04), and three gourami iridovirus cases, four cichlid iridovirus cases and one poeciliid iridovirus case have been found since the survey (2009 to 2010).

With the exception of the studies undertaken by Go et al. (2006) and Jeong et al. (2008b), no information is available on the prevalence of megalocytiviruses in cichlids, gouramis and poeciliids. The 1999 IRA recognised that prevalence varies between source populations.

Studies by Go et al. (2006) on fish collected from two pet shops indicate that post-quarantine megalocytivirus prevalence is in the order of 8–56 per cent. Considering the annual volume of gouramis imported into Australia, the likelihood of release for gourami iridovirus is high.

In the absence of information on the prevalence of megalocytiviruses in cichlids and poeciliids and taking into account the volumes imported, the likelihood of release post-quarantine for cichlid and poeciliid iridoviruses is also high.

Ranaviruses

GV–6 has been isolated from healthy fish, suggesting the occurrence of a carrier state (Hedrick and McDowell 1995). Studies conducted in Europe using bath exposure have shown that angelfish, zebrafish (*Danio rerio*) and gouramis can be susceptible to GV–6 and can be potential carriers. Goldfish (*Carassius auratus*) and carp (*Cyprinus carpio*) were found not to be susceptible to GV–6/DFV.

European studies have shown that cichlids (angelfish), gouramis (pearl gourami), poeciliids (guppies) and zebrafish are possible carriers of ESV/ECV. Goldfish and carp were shown not to be susceptible to any piscine iridoviruses.

Fish cohabiting with frogs and toads in ponds can become infected with amphibian ranaviruses [for example, Redwood Park virus and Stickleback virus, FV3-like virus in American bullfrog (*Rana catesbeiana*) and *Gnathopogon* spp.]. Goldfish are commonly reared in outdoor ponds and

if not biosecure, wild frogs and toads could enter these ponds and act as vectors for the transmission of the virus to fish in other ponds.

RTRV has been reported from amphibians in China and amphibians, goldfish and food fish [marble goby (*Oxyeleotris marmoratus*)] in Thailand. Of the goldfish imported into Australia, 43 per cent is imported from China and 6 per cent from Thailand (Department of Agriculture data 2008). Some of these goldfish may be used to stock garden ponds or be used as broodstock, and the progeny reared in ponds that are not secure from amphibians.

There is some evidence of infection of goldfish with RTRV under natural conditions. The prevalence of RTRV in goldfish is unknown.

A survey of imported ornamental fish conducted in Europe using cell culture and PCR did not reveal the presence of ranaviruses in any of the samples (Ariel 2009), indicating ranavirus prevalence in fish imported into Australia is likely to be negligible.

Goldfish iridoviruses

GFV–1 and GFV–2 were found only in healthy fish suggesting the existence of a carrier state (Berry et al. 1983). There is no information available regarding prevalence.

There is only a single report each of natural infections of GFV in goldfish, GV–6 in guppy and RTRV in goldfish, despite both fish species generally being subject to more study than many other ornamental fish species. The likelihoods of release associated with importation of poeciliids and goldfish would be much less for ranaviruses and goldfish iridoviruses compared to megalocytiviruses.

There are no reports of natural infections of ranaviruses in zebrafish, cichlids or gouramis, although they have been infected via experimental bath exposure.

Surveys conducted in the Europe did not show any ranavirus in imported ornamental fish, suggesting that the prevalence is negligible. The likelihoods of release for ranaviruses and goldfish iridoviruses would be very low.

Conclusions

Based on the considerations in Section 5.1, the likelihood of release of iridoviruses of concern associated with freshwater ornamental fish is estimated as:

Megalocytiviruses	Cichlids, gouramis and poeciliids	–	high
Piscine ranaviruses	Cichlids and gouramis, poeciliids and zebrafish	GV–6 ESV/ECV	very low
Amphibian ranavirus	Goldfish	RTRV	very low
Goldfish iridoviruses	Goldfish	GFV–1/2	very low

5.2 Exposure assessment

The exposure assessment component of this assessment determined, for each iridovirus of quarantine concern, the likelihood that a domestic susceptible host population was exposed to potentially infected or contaminated ornamental fish or associated materials imported into Australia.

This risk assessment considered the potential exposure of the following three exposure groups, and determined the likelihood of exposure for each:

- fish populations within the ornamental fish industry
- farmed foodfish populations
- susceptible host species in natural waters. Note that native amphibians in the natural environment may be exposed to amphibian ranaviruses carried by ornamental fish similar to fish in natural waters.

In determining each exposure likelihood, various risk factors associated with all pathways were taken into account, from the point of release from quarantine detention. The three key exposure groups and pathways by which they could become exposed to an iridovirus of quarantine concern are depicted in Figure 3 (section 2.4.2).

The 1999 IRA exposure assessment considered the likelihood of local ornamental fish being exposed to a dose of virus sufficient to cause infection and the likelihood of agent establishment, that is, occurrence of an index case of infection, spread from an index case to other fish and disease establishment in the exposed population(s). Consistent with the department's risk assessment method, in this IRA, the exposure assessment considered only the likelihood of exposure of local fish to a dose of virus sufficient to cause infection. The likelihood of subsequent establishment or spread is covered later in the consequence assessment.

5.2.1 Exposure group 1—ornamental fish industry

1999 IRA—key considerations

For iridoviruses of quarantine concern associated with freshwater ornamental fish, the 1999 IRA determined the likelihood of ornamental fish being exposed to a dose of virus sufficient to cause infection and for the virus to spread to other fish to be *low to moderate*. The 1999 IRA exposure assessment considered the likelihood of agent establishment (that is occurrence of an index case of infection, spread from an index case to other fish, and disease establishment in the exposed population/s). Consistent with the department's current risk assessment methodologies, the exposure assessment in this report considers the likelihood of exposure; the likelihood of subsequent establishment or spread is covered in the consequence assessment component of this report.

In determining the likelihood, in general terms, of exposure of the ornamental fish industry to a pathogenic agent, the 1999 IRA based its conclusions on the following criteria:

- the host range and relative numbers of these species traded within the industry
- the likelihood of the agent being detected (which is related to the prevalence of subclinical infection)
- the transmissibility of the agent.

In considering these criteria, the 1999 IRA took into account the following:

Distribution/exposure pathways

Freshwater ornamental fish enter the country via fish importers who are, in most cases, also wholesalers. Most of these fish are sold to retailers who in turn sell primarily to hobbyists. Retailers also sell a very small number of fish to commercial, semi-commercial and backyard breeders, who in turn sell stock back to wholesalers and to a lesser extent, direct to retailers and hobbyists. A very small number of fish (mainly marine) may go into public aquariums for display. For the purposes of the 1999 IRA, all these groups formed the ornamental fish industry.

Epidemiological factors

Ornamental fish species that pose the greatest risk are those more likely to survive the disease and shed the agent into the environment over a prolonged period. Although it should be noted that fish showing clinical signs of disease are actively disseminating the agent and more likely to transmit disease than subclinically infected fish.

Subclinically infected fish are less likely to be intercepted along the supply chain and as a result more likely to be supplied to end-users. Pathogenic agents may be carried by ornamental fish species without causing clinical signs of disease and consequently there would be a high likelihood of such carrier fish being transferred along the supply chain to end-users.

2010 IRA—new considerations

In addition to information presented in the 1999 IRA, the department considers the following relevant to the estimation of the likelihood of exposure:

Distribution/exposure pathways

- Following the release from post-arrival quarantine by the department, fish are transferred to wholesale facilities for subsequent distribution to retailers, where they are held captive in aquariums. Imported ornamental fish may be mixed with locally produced ornamental fish at wholesale or retail centres before being distributed to various end-users. If not mixed at distribution centres, imported freshwater ornamental fish may be transported to other retailers and hobbyists resulting in direct exposure of local freshwater ornamental fish to potentially infected imported ornamental fish. Exposure of susceptible local host populations to an iridovirus of quarantine concern is therefore most likely to occur within the ornamental fish industry.

- The vast majority of imported fish are destined for home aquaria. Data on relative volumes of live ornamental fish directed towards each exposure group were unavailable; however, the vast majority of imported ornamental fish would be directed towards the ornamental fish industry. Around 5 per cent of imported ornamental fish go directly to retailers with quarantine approved places, half of which are freshwater ornamental species (J. Patrick, PIAA, pers. comm. December 2005). The subsequent distribution of imported ornamental fish from these retailers is similar to the distribution from retailers supplied by wholesalers.

- Native species such as barramundi (*Lates calcarifer*) – which can be held in freshwater and saltwater–and Murray cod are also kept in aquariums by hobbyists. As carnivores, these species are fed feeder fish (low value fish bought for the purpose of feeding carnivorous fish purchased from wholesalers or retailers, and are sometimes released to the wild when they reach an unmanageable size (K. Weaver, Fisheries Victoria, pers. comm. October 2005). Feeder fish are expected to comprise locally produced and

imported low-cost species such as goldfish, rosy barbs (*Puntius conchonius*, Family Cyprinidae) and guppies. Although cichlids, gouramis and zebrafish are less likely to be used this way, gouramis may be fed to carnivorous fish held in hobbyist aquariums and may be emerging as a feeder fish in the ornamental fish trade (M Landos, Future Fisheries Veterinary Service, pers. comm. June 2007).

Epidemiological factors

Megalocytiviruses

Go and Whittington (2006) demonstrated the potential for gouramis to harbour iridovirus without exhibiting disease signs at least 28 days post-infection. It is reasonable to assume that susceptible ornamental fish species may also harbour other iridoviruses of quarantine concern without showing signs of clinical disease.

Megalocytiviruses have been reported from cichlids, gouramis and poeciliids under natural conditions.

Ranaviruses

Piscine ranaviruses have been transmitted experimentally to poeciliids (guppies), cichlids (angelfish), gouramis (pearl gourami) and zebrafish via bath exposure, suggesting that freshwater ornamental fish species may be naturally susceptible to piscine ranaviruses (GV–6, ESV/ECV).

Amphibian ranaviruses (RTRV) have been reported from goldfish in Thailand, although an epidemiological link between RTRV in frogs and fish has not been demonstrated.

Poeciliids, gouramis, cichlids, goldfish and zebrafish continue to be widely traded both internationally and in Australia.

Goldfish iridoviruses

There is only one report of goldfish iridovirus in goldfish.

Conclusions

Based on the considerations above, the likelihood of local ornamental fish (exposure group 1) being exposed to an imported freshwater ornamental fish infected with an iridovirus of concern is estimated to be:

Megalocytiviruses	Cichlids, gouramis and poeciliids	–	moderate
Piscine ranaviruses	Cichlids and gouramis, poeciliids and zebrafish	GV–6 ESV/ECV	moderate moderate
Amphibian ranavirus	Goldfish	RTRV	moderate
Goldfish iridoviruses	Goldfish	GFV–1/2	moderate

5.2.2 Exposure group 2—farmed foodfish

1999 IRA—key considerations

The 1999 IRA did not identify farmed freshwater foodfish as an exposure group and thus no likelihoods were estimated. The significance of this exposure group has since been identified in

this assessment as a result of the outbreak of iridovirus in farmed Murray cod (Lancaster et al. 2003) and the transmission studies undertaken by Go and Whittington (2006).

2010 IRA—new considerations

In determining the likelihood of exposure for each of the four iridoviruses of quarantine concern, the department considers the following to be relevant:

Distribution/exposure pathways

Feeding of farmed foodfish broodstock with live or dead ornamental fish represents a potential pathway for exposure of farmed foodfish. There are indications that ornamental fish may be used (albeit rarely) as food in hatcheries, where farmers may condition their broodstock by feeding live ornamental fish before commencement of the breeding season (B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005). However, industry feedback to the department during visits to commercial foodfish hatcheries suggests feeding dead or live imported ornamental fish purchased from wholesalers or retailers would be rare.

Production of freshwater foodfish in Australia, including Murray cod, has increased over the last decade.

Ornamental species used as food for farmed foodfish broodstock are likely to be those low cost species, that is those widely produced in low input, pond-culture systems in Australia (such as goldfish and poeciliids). However, use of locally produced gouramis as feeder fish for foodfish kept in aquariums (for example, Murray cod) has been reported, suggesting that low input, pond-cultured locally bred gouramis may also emerge as a feeder fish species for foodfish broodstock. Feeding of zebrafish or cichlids has not been reported.

An iridovirus thought to be a minor variant of ISKNV or DGIV (genus *Megalocytivirus*) was detected in association with mortality in farmed Murray cod (Lancaster et al. 2003). It is not known how the virus was introduced to the farm but it may have been through the use of ornamental fish as food for broodstock. This may have occurred in the hatchery supplying fingerlings to the farm or broodstock on the farm concerned. Other fish species commercially farmed in freshwater in Australia and potentially at risk of exposure through this pathway include barramundi, silver perch (*Bidyanus bidyanus*) and Barcoo grunter– marketed as jade perch (*Scortum barcoo*).

Queensland is the only state that has government controls or prohibitions on the use of live ornamental fish as food in freshwater fish farm operations. It is an offence under Queensland's *Animal Care and Protection Act 2001* to feed a live fish to another fish. However, locally bred ornamental fish are kept together with foodfish broodstock in open ponds resulting in ornamental fish unintentionally being eaten by foodfish broodstock (B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005). The Western Australian ornamental fish and commercial aquaculture activities are managed under the *Fish Resources Management Act 1994* (FRMA), which does not prohibit cohabiting of multiple species in the same tank or the use of live fish as feed. Go and Whittington (2006) showed that iridovirus can be transmitted to Murray cod cohabiting with infected gouramis. Therefore, the practice of rearing ornamental fish with foodfish species or the feeding of broodstock with ornamental fish may result in the direct spread of infection via water, ingestion or contact.

Epidemiological factors

The capacity for gouramis to harbour iridovirus without showing clinical signs of disease has been demonstrated. It is thus reasonable to assume that susceptible ornamental fish species may also harbour other iridoviruses of quarantine concern without showing clinical signs of disease (for example, GV–6 in guppies).

Experimental transmission studies have shown that horizontal transmission via cohabitation of infected fish, infected water, ingestion of infected excreta, or cannibalism of dead fish is likely. Vertical transmission has not been demonstrated.

Conclusions

Based on the considerations above, the likelihood of local farmed foodfish (exposure group 2) being exposed to an imported freshwater ornamental fish infected with an iridovirus of concern is estimated to be:

Megalocytiviruses	Cichlids, gouramis and poeciliids	–	very low
Piscine ranaviruses	Cichlids and gouramis, poeciliids and zebrafish	GV–6 ESV/ECV	very low very low
Amphibian ranavirus	Goldfish	RTRV	very low
Goldfish iridoviruses	Goldfish	GFV–1/2	very low

5.2.3 Exposure group 3—susceptible host species in natural waters

1999 IRA—key considerations

For iridoviruses of quarantine concern associated with freshwater ornamental fish, the 1999 IRA determined the likelihood of susceptible fish in Australian natural waters being exposed to a dose of virus sufficient to cause infection and for the virus to spread to other fish to be *extremely low*.

In determining the likelihood, in general terms, of agent exposure to fish in natural waters through importation of ornamental fish, the 1999 IRA based its conclusions on the following criteria:

- the likelihood that infected fish will enter and survive for prolonged periods in natural waters
- the ability of the agent to survive in the environment outside the host
- presence of susceptible host species in natural waters.

The 1999 IRA exposure assessment considered the likelihood of agent establishment, (that is occurrence of an index case of infection, spread from an index case to other fish, and disease establishment in the exposed population/s). Consistent with Biosecurity Australia's current risk assessment methodologies, in this review, the exposure assessment considers the likelihood of exposure; the likelihood of subsequent establishment or spread is covered in the consequence assessment component of this review. In considering these criteria, the 1999 IRA took into account the following:

Distribution/exposure pathways

- The two main ways for live freshwater ornamental fish to be released into natural waters are via people deliberately releasing unwanted and sick fish into natural waters, a practice termed the 'Christmas syndrome' in the ornamental fish trade, and usually involves inexperienced or first-time hobbyists releasing ornamental fish (for example, goldfish), and fish that escape from earthen or ground-level ponds (either grow-out ponds in breeding facilities or hobbyists' garden ponds) near or with a direct connection to natural waters, as a result of vandalism, or accidental or inadvertent breakdown in holding systems (for example, during floods).
- Only a small proportion of imported ornamental fish is estimated to reach local facilities that breed ornamental fish (probably for use as broodstock). It is unlikely that these fish would escape into natural waters, but if infected, they might infect other fish in breeding facilities and grow-out ponds and these infected pond fish may in turn find their way into natural waters. Intensive production conditions may lead to amplification of pathogen numbers.
- Goldfish and poeciliids were the only ornamental fish species considered in the 1999 IRA to be produced in Australia in open pond culture systems.
- Disease may also spread from pond culture operations to natural waters through activities of piscivorous (fish eating) birds and escape or release of susceptible feral or native fish that have been introduced into ponds as eggs or fry.
- The likelihood of fish surviving to form a self-maintaining population depends on many factors, including the 'propagule pressure' of any release event. A propagule is the unit, or number of individuals, involved in an invasion event. Propagule pressure is the effect on the likelihood of successful invasion of increasing or decreasing the size and the number of propagules (Arthington et al. 1999). One important factor determining propagule pressure is the number of individual fish entering the environment in a given release event. For example, if 100 fish escape into a natural waterway from a single release event at one site (for example, flooding of an earthen pond), the propagule pressure is greater than that associated with 100 separate release events by hobbyists each releasing one fish into natural waters at different sites.
- The likelihood of introduction of pathogenic agents through escapes from breeding facilities would be higher than the likelihood associated with 'Christmas syndrome' releases because the volume of fish reared in breeding facilities is much higher compared to hobbyist aquariums.
- Arthington et al. (1999) identified cichlids, cyprinids (Family Cyprinidae including carp, goldfish, barbs, danios and rasboras) and poeciliids as groups having 'high to very high' likelihoods of establishment in Australian waters.
- The entry of goldfish and poeciliids into natural waters constitutes a potentially significant pathway for disease establishment.

The 1999 IRA also took into account the following in assessing the iridovirus-specific likelihood of agent 'establishment' in fish in Australian natural waters:

- Although extensively bred in the ornamental fish industry, cichlids and gouramis are not raised in ponds and therefore not associated with significant pathways by which infected fish may enter natural waters.

2010 IRA—new considerations

In addition to information presented in the 1999 IRA, the department considers the following relevant to an estimation of the likelihood of exposure.

Distribution/exposure pathways

Release of imported ornamental fish into natural waters represents a direct (potential) pathway for exposure of free-living populations of susceptible host species. Susceptible species would include native Australian and introduced fish species (for example, trout, salmon and redfin perch) in the natural environment and in the case of ranaviruses, species of amphibians.

Lintermans (2004) reported 22 of 34 alien species established in Australia as originating from the ornamental fish industry and identified 'bait bucket introductions', discarding of unwanted ornamental fish, escape from aquaculture facilities, escape from ponds and dams, and deliberate release for cultural/religious reasons as possible means of establishing free-living populations of ornamental fish. 'Bait bucket introductions' are where anglers who use bait fish (for example, goldfish), discard excess fish either into the waterways they are fishing, or into local dams and ponds to provide bait for subsequent fishing trips.

Escape from pond culture:

- Small-scale ornamental gourami pond culture occurs in Australia, albeit to a lesser extent than that associated with goldfish and poeciliids (D. Ogburn, New South Wales Department of Primary Industries, pers. comm. August 2005, B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005). Imported ornamental fish may be introduced into these ponds as broodstock when needed to improve their stock genetically, although such introductions would be infrequent in commercial operations. For example, one major ornamental fish breeder advised that they purchased new imported stock once in five years. Open pond rearing of ornamental cichlids is not known to occur in Australia (B. Sambell, Aquaculture Association of Queensland Inc., pers. comm. September 2005). As such, there is a higher likelihood of goldfish, poeciliid and (to a much lesser extent) gourami associated iridoviruses being introduced into natural waters through fish escape from ponds compared to cichlid iridoviruses. Ornamental cichlids are most likely to enter natural waters as a result of the discarding of unwanted fish by hobbyists (M. Lintermans, UC, pers. comm. October 2008).

- Wild frogs and toads may cohabitate with ornamental fish reared in garden ponds and become infected with amphibian ranaviruses (for example, RTRV). These amphibians may subsequently move from one pond to another and act as vectors of virus spread.

- Arthington et al. (1999) identified cichlids, cyprinids (Family Cyprinidae which includes carp, goldfish, barbs, danios and rasboras (*Trigonostigma* spp.) and poeciliids as groups having 'high to very high' likelihoods of establishment in Australian waters. Members of the Osphronemidae (which includes the subfamilies Luciocephalinae and Macropodinae) were identified as having 'moderate to very high' likelihood of establishment in Australian waters. The categorisation of risk species by Arthington et al. (1999) was based on several factors including reported occurrence of wild populations in Australia.

The cichlids, jewelfish (*Hemichromis bimaculatus*), blue acaras (*Aequidens pulcher*), Jack Dempseys (*Cichlasoma octofasciatum*), and hybrid cichlids (*Labeotropheus/Pseudotropheus* cross), and the three-spot gourami have been reported in the wild in Australia since 2000 (Raadik 2003; Raadik 2004). There are no reports of zebrafish establishment in the wild.

- All state and territory governments have legislative controls on aquaculture production, including the rearing of fish in open ponds. For example, in South Australia, exotic species can be farmed only under permit, with growers required to have an approved strategy to prevent escape of stock and licences issued only if ponds are above the one in 100-year flood level to avoid escape of stock to natural waters. Under Queensland's aquaculture regulations, there are similar controls on aquaculture including ornamental fish production and aquaculturists have an environmental code of best practice that includes prevention of escape. These legislative controls and codes of practice would reduce the potential for exposure of fish in natural waters to imported fish carrying exotic pathogenic agents, although the level of risk reduction depends on the degree of enforcement of the legislation by state and territory authorities, and the level of compliance by ornamental fish producers. It should be noted that the states and territories have field based officers that monitor legislative compliance. For example, the South Australian Government has 0.1–0.2 of a staff member from Fishwatch [Department of Primary Industries and Resources of South Australia (PIRSA) Fisheries compliance section] allocated to control ornamental fish industry issues (M Deveney, PIRSA Aquaculture, pers. comm. March 2007).

Use of ornamental fish as fishing bait:

- A 2002 survey of bait use in the Australian recreational fishing sector identified that ornamental fish may be used as bait for recreational fishing (assumed to include use in fishing for freshwater fish species) (Kewagama Research 2002). The report indicated that freshwater fish species used as bait were sourced from either bait shops or were caught by fishers. With respect to ornamental fish species, only the use (albeit it very rare) of guppies was specifically reported, with one report from Victoria and one from Queensland of guppies being sourced from bait suppliers, and two reports from Victoria of guppies being personally caught for use as bait. Respondents reported using both live and dead fish as bait (Kewagama Research, unpublished data). There was no indication that cichlids, goldfish, gouramis or zebrafish were being used as recreational fishing bait.

- Chong et al. (2005) alluded to the practice by some anglers of purchasing live ornamental fish (some of which may be imported), especially goldfish, for use as bait. Despite specific questioning relating to goldfish during the Kewagama Research survey, there were no reports of goldfish being used as bait. This does not mean that goldfish are not used as bait, but that if it does occur, then it is at very low levels. Several state government officials have since indicated that there was anecdotal evidence of the use of goldfish as bait (D. Ogburn, New South Wales Department of Primary Industries, pers. comm. August 2005, T. Hawkesford, Queensland Department of Primary Industries, pers. comm. September 2005, K. Weaver, Department of Fisheries Victoria, pers. comm. October 2005, M. Deveney, PIRSA Aquaculture, pers. comm. August 2005.), as well as infrequent use of guppies, loaches and other small fish (M. Deveney, PIRSA Aquaculture, pers. comm. August 2005). There has been no specific mention of the use of cichlids, gouramis or zebrafish as bait. There are reports of Nile tilapia being caught from natural

waters and used as bait. Nile tilapia are declared a noxious species and are not permitted to be imported into Australia.

- Legislation in Australian states and territories, other than Western Australia, prohibits the release of live ornamental fish, including for use as bait. Legitimate release of translocated fish can occur only with government approval. There is no government control on the use of dead fish as bait, except in Tasmania where the Inland Fisheries (Recreational Fishing) Regulations 1999 prohibits the use of live or dead fish as bait in all inland waters other than estuarine waters (Raadik 2001).
- Although changes to legislation have made it illegal to use live fish as bait in freshwater in many states and territories, the practice still occurs, albeit rarely (T. Hawkesford, Queensland Department of Primary Industries, pers. comm. September 2005, K. Weaver, Department of Fisheries Victoria, pers. comm. October 2005). In addition, anglers who use live fish as bait are prone to discarding excess fish either into the waterways they are fishing, or into local dams and ponds to provide bait for subsequent fishing trips. These 'bait bucket introductions' provide the potential for exposure of free living fish to imported fish and exotic pathogenic agents that they may harbour (Lintermans 2004).

Epidemiological factors

The capacity for gouramis and guppies to harbour iridovirus without exhibiting clinical signs of disease has been demonstrated. Therefore, it is reasonable to assume that susceptible ornamental fish species may also harbour other iridoviruses of quarantine concern without showing clinical signs of disease.

Conclusions

Based on the considerations above, the likelihood of susceptible host species in natural waters (exposure group 3) being exposed to an imported freshwater ornamental fish infected with an iridovirus of concern is estimated to be:

Megalocytiviruses	Cichlids, gouramis	–	very low
	Poeciliids		low
Piscine ranaviruses	Poeciliids	GV–6	low
		ESV/ECV	low
	Cichlids and gouramis	GV–6	very low
		ESV/ECV	very low
	Zebrafish	GV–6	negligible
		ESV/ECV	negligible
Amphibian ranavirus	Goldfish	RTRV	low
Goldfish iridoviruses	Goldfish	GFV–1/2	low

5.3 Consequence assessment

Likely consequences were assessed separately for each exposure group. A conclusion was reached on the likelihood of establishment or spread and the level and magnitude of any resulting biological, economic and environmental impacts associated with the outbreak scenario. The outbreak scenario used for each exposure group was that the agent established or spread in exposed populations and spread further to other natural and captive populations of susceptible host species in Australia.

5.3.1 Likelihood of establishment or spread

In determining the likelihood that the selected outbreak scenario would occur, consideration was given to the factors that influence the likelihood of agent establishing or spreading to other susceptible host populations.

The interaction between host, environmental and agent factors is critical to the likelihood of agent establishment or spread. Information on these factors in relation to iridoviruses of freshwater ornamental fish was limited. Where available, such information was considered in likelihood estimations. Scientific judgements were made where information was lacking.

1999 IRA—key considerations

With respect to the likelihood of establishment or spread, the 1999 IRA identified the following general points:

Epidemiological factors

- Ornamental fish species that pose the greatest risk are those more likely to survive the disease and shed the agent into the environment over a prolonged period.
- Subclinically infected fish are less likely to be intercepted along the supply chain and are thus more likely to be supplied to end-users as they do not show clinical signs of disease.
- International trade in ornamental fish has been implicated in the spread of several aquatic animal diseases, including to aquatic animals in Australian natural waters, for example, EHNV (Langdon et al. 1986; Langdon and Humphrey 1987).
- The likelihood of disease establishment in breeding, wholesale or retail facilities depends on the type of facilities, its size, the range of fish species held and management practices.
- If a disease were to occur in facilities supplying a significant part of the hobby sector or many breeders, then it could spread widely. Such a scenario constitutes a significant potential pathway for a pathogenic agent to establish within the ornamental fish industry.
- Disposal of fish and other wastes from ornamental industry facilities (other than importer facilities) is not considered to constitute a significant pathway for establishment or spread, because potentially infected material is treated through normal waste management (that is sewerage) practices. In addition, there is a dilution factor provided by domestic waste disposal systems.
- The consequence assessment component of the 1999 IRA considered only impacts. Determination of the likelihood of establishment or spread was incorporated into what was effectively an overall likelihood of release and exposure.

Other factors

- Most imported ornamental fish are destined for home aquariums, from which pathogenic agents are most unlikely to spread. A small number of fish would be expected to go to backyard breeders.
- Industry has adopted a code of practice that includes adoption of procedures to minimise the opportunity for transferring pathogenic agents, so that overtly diseased fish are unlikely to be transferred between industry sectors.

Likelihood of establishment or spread from exposure group 1—ornamental fish industry

1999 IRA—key considerations

The 1999 IRA took into account the following iridovirus specific-information in assessing the likelihood of agent establishment in the ornamental fish industry:

- Cichlids and gouramis are susceptible to iridoviruses and are commonly traded in Australia.
- Horizontal transmission via ingestion of infected excreta and cannibalism of dead fish is likely.
- An iridovirus infection in a gourami farm in Florida (United States) was reported, indicating the ability of the virus to establish and spread in fish farms.

2010 IRA—new considerations

In addition to information presented in the 1999 IRA, the department considers the following relevant to an estimate of the likelihood of establishment or spread associated with exposure of a population of ornamental fish within the Australian ornamental fish industry.

Likelihood of agent establishment or spread in a local fish population within the ornamental industry

- Go and Whittington (2006) demonstrated the potential for gouramis to harbour megalocytiviruses without exhibiting disease signs for at least 28 days post-infection. Go and Whittington (2006) also demonstrated the ability of iridovirus from gouramis to be transmitted via water. Jeong et al. (2008b) demonstrated using 2-step PCR that poeciliids, gouramis and cichlids can be subclinical carriers of megalocytiviruses. Thus, it is reasonable to assume that susceptible ornamental fish species may harbour megalocytiviruses without showing clinical signs of disease and the viruses be similarly transmitted under industry holding conditions.
- Once released from quarantine, imported ornamental fish may continue to shed virus and infect other imported and locally produced ornamental fish through sharing of display tanks, equipment or water in wholesale or retail facilities before being sold to other hobbyists and breeders. Tanks, equipment and water are unlikely to be sterilised between batches, further increasing the likelihood of disease establishment or spread in these populations.
- Practices of mixing different species of local and imported freshwater ornamental fish, mixing different species in retail shops and home aquaria, inadequate disinfection

procedures at retail and wholesale distribution centres and use of ornamental fish species (such as goldfish and guppies) as feeder fish for carnivorous aquarium species such as Murray cod (including potential risks associated with transport water) are likely to result in spread of iridovirus from imported carrier fish to other susceptible species, both imported and domestically produced.

- O'Sullivan et al. (2008) classified hobbyists into following three subcategories: serious hobbyists who are likely to spend more than $1000 per year on fish and be involved in hobby organisations and regularly show their animals, moderate hobbyists who are likely to spend between $100-1000 per year but are not involved with selling of animals and include businesses which display aquatic animals for their customers or staff enjoyment and irregular hobbyists who may occasionally purchase fish and are likely to spend less than $100 a year. Serious hobbyists may breed fish and will sell fish to or swap for accessories, feeds or other animals with other hobbyists or retailers. Hobby breeding and swapping sales may result in translocation of both exotic and native species, and associated disease spread.

- Local ornamental fish breeders purchase imported fish as broodstock. Thus, it is likely that imported ornamental fish species are occasionally introduced into open ponds in local farms potentially leading to the establishment or spread of disease in local ornamental fish broodstock.

- If an exotic pathogenic agent were to become established in local ornamental fish broodstock via imported ornamental fish, it is likely that the infection would spread to their offspring via cohabitation. Subsequently, these fish might be sold as feeder fish and represent a potential pathway for disease spread from the ornamental fish industry to hobbyist aquariums and foodfish farms. In addition to more commonly fed species (for example, goldfish and poeciliids), there is anecdotal evidence that locally produced gouramis are sold as feeder fish for carnivorous native species (such as barramundi and Murray cod) kept in hobbyist aquariums (M. Landos, Future Fisheries Veterinary Service, pers. comm. June 2007).

- Reports of GV–6 is limited to one publication in 1995 (Hedrick and McDowell 1995) where the virus was isolated from healthy fish. A closely related ranavirus, DFV, has been isolated from a marine ornamental fish, doctorfish (*Labroides dimidiatus*) (Hedrick and McDowell 1995). Doctorfish is not listed in the Department of the Environment Permitted Species List.

- Challenge trials have shown that gouramis, cichlids and zebrafish are also susceptible to GV–6 via bath exposure (Bang Jensen 2009). Thus, gouramis, cichlids and zebrafish could carry the virus, potentially leading to virus establishment or spread in these species. Guppies have been shown to be asymptomatic carriers of GV–6 (Hedrick and McDowell 1995) and goldfish and carp shown not to be susceptible (Bang Jensen 2009).

- Similarly, poeciliids, gouramis, cichlids and zebrafish have also been shown to be susceptible to ESV/ECV, but goldfish and carp were not (Bang Jensen 2009).

- There is one report of RTRV in diseased goldfish from Thailand (Kanchanakhan et al. 2003) suggesting that goldfish may be susceptible to amphibian ranaviruses. However, there is no information available on the epidemiology of RTRV infection in goldfish.

- In Europe, guppies challenged with FV–3 via bath exposure were shown not to be susceptible, as no virus was isolated from dead fish.
- Reports of goldfish iridoviruses are restricted to a single publication on the isolation of GFV–1 and GFV–2 from primary cell culture derived from healthy fish. Goldfish iridoviruses have been isolated only from goldfish and there have been no reports of disease in goldfish or any other species. Without further information, valid assumptions cannot be made on pathogenicity, host specificity or transmission of these viruses.

Likelihood of agent spread to other susceptible host populations once established or spread in the exposed ornamental fish population

Release of ornamental fish into natural waters:

- Release of live ornamental fish into natural waters constitutes the most significant pathway by which iridoviruses of quarantine concern may be introduced into the natural environment. Ornamental fish may enter natural waters by either deliberate release or inadvertent escape from ground level ponds during flooding.
- The high likelihood of iridovirus spread within the ornamental fish industry from a given imported species to a range of other susceptible species (as previously discussed) means that the species responsible for spread of virus from the industry to, for example, natural waters, need not necessarily be as a result of escape or release of an imported species, but rather due to a species more likely to escape or be released into natural waters.
- Deliberate release can occur by hobbyists releasing unwanted fish into natural waters ('Christmas syndrome') or release of unused bait by recreational fishers. Irregular hobbyists, compared to serious or moderate hobbyists, pose a greater risk of disease spread to wild fish and other susceptible populations through inappropriate disposal of sick/dead or unwanted fish.
- Information available on the numbers of ornamental pond-culture facilities in Australia is anecdotal. Commercial pond farms that are licensed under the state and territory legislation are much less likely to pose a significant risk, as regulation includes managing the risk of fish escaping.
- Goldfish would have a higher likelihood of being released into natural waters than other ornamental fish species as they are commercially reared in ground level ponds, kept in garden ponds and are most associated with release into natural waters by people ('Christmas syndrome'). Goldfish may also to be used as recreational fishing bait. Poeciliids are more likely to be released into natural waters than cichlids and gouramis due to their higher likelihood of being pond cultured and being used as bait for recreational fishing. The bait and berley survey (2000) indicates however that if this practice occurs, it occurs at very low levels. Similarly, gouramis are more likely to be introduced into natural waters than cichlids due to the higher likelihood that gouramis are pond cultured and they may be used as bait. Zebrafish are unlikely to be pond cultured or be used as bait.
- Native species such as barramundi and Murray cod are also kept in aquaria by hobbyists for ornamental purposes. As carnivores, these species are fed feeder fish purchased from wholesalers or retailers, and are sometimes released to the wild or put back into fish farms when they reach an unmanageable size (K. Weaver, Department of Fisheries, Victoria, pers. comm. October 2005, B. Sambell, Aquaculture Association of Queensland

Inc., pers. comm. January 2006). This is a potential pathway for the spread of disease from the ornamental fish industry to fish in natural waters or farmed foodfish.

Survival of fish released into natural waters:

- Survival of fish released into natural waters and their ability to form self-maintaining populations would add to the likelihood of an iridovirus of quarantine concern establishing in receiving waters. Arthington et al. (1999) identified several ornamental fish families as having a 'moderate to very high' likelihood of surviving and forming self-maintaining populations in Australia, including cichlids, cyprinids and poeciliids. Similarly, members of the Osphronemidae (which includes the subfamilies Luciocephalinae and Macropodinae) were identified as having 'moderate to very high likelihood of establishment in Australian waters'. Categorisation of risk by Arthington et al. (1999) was based on several factors including reported occurrence of wild populations in Australia.
- The likelihood that escaped fish establishing self-maintaining populations in natural waters would depend on a range of factors, including the biology of the species and the environment into which they are introduced. Cichlids, goldfish, gouramis and poeciliids have established wild populations in Australia.

Exposure of susceptible host species in natural waters:

- Corfield et al. (2008) describes the location of feral populations of cichlids, cyprinids including goldfish, gouramis and poeciliids found in Australia (Appendix D), although some introduced species may persist for some years but fail to establish in the natural environment to which they are introduced (R. McKay, Chillagoe Museum, pers. comm. March 2010). These wild populations are predominantly in urban/peri-urban areas, confined to the warmer latitudes of Australia in the case of tropical species (cichlid, gourami and poeciliid species), but overlap those latitudes that are home to native and introduced finfish species which may be susceptible to infection, including Murray cod and rainbow trout (*Oncorhynchus mykiss*). The Murray cod occurs naturally in the waterways of the Murray-Darling Basin (Australian Capital Territory, South Australia, New South Wales and Victoria) and is known to live in a wide range of warm water habitats. Murray cod can live in temperatures varying from 19–34 °C although the optimum temperature is reported to be 20–25 °C. Translocated populations currently exist in New South Wales and Victoria and are maintained by the release of hatchery bred fish.
- Rainbow trout and brown trout (*Salmo trutta*) are the most common freshwater trout species grown in Australia. Brown trout are produced primarily for state based recreational fishing stock enhancement programs in the cooler, upland catchments of New South Wales, Victoria and Tasmania. Rainbow trout are produced on a larger scale for human consumption and to some extent, for recreational purposes, including stock enhancement of public and private waters and on-farm fish-out operations. Although trout are a cold water species (5 °C to <20 °C) (Moloney 2001), they have a wide habitat range and are reported to feed in water temperatures up to 23 °C or higher depending on the species, thermal history and life stage (Moloney 2001). High mortality rates of trout generally occur at between 26 °C and 27 °C (Morrissy 1973). Rainbow trout reportedly have slightly lower maximum critical thermal maxima than brown trout (Moloney 2001). Rainbow trout show reduced feeding and therefore lower growth rates in water temperatures above 20 °C. Further, (Morrissy 1973) showed that the rainbow

trout maintained at the South West Freshwater Research and Aquaculture Centre (Pemberton, Western Australia) had an increased ability to tolerate higher temperatures (up to 23 °C) than stocks in eastern Australia. Although poeciliids, cichlids and gouramis are tropical species, there is overlap of latitudes where these species could co-exist for some time, including in rainbow trout stocked impoundments or streams. Guppies (poeciliids) have wide salinity tolerances, but require fairly warm temperatures (23-24 °C) and quite vegetated water for survival. However, this species has been found in many temperate countries so its actual temperature tolerance is much greater than Fishbase suggests. There is evidence that guppies can survive water temperatures as low as 20 °C (Corfield et al 2008).

- Based on the geographical pattern of its spread, scientists have speculated that SCRV may have been introduced into the United States with the movement of guppies, which were reared in ground level ponds and may have escaped into natural waters (Grizzle and Brunner 2003; Hedrick and McDowell 1995), although the available literature indicates that the origin of SCRV in the United States is far from resolved. Although SCRV can infect multiple species, there is no evidence to suggest that ornamental fish species would be susceptible.

- GV–6 is taxonomically placed under the SCRV group of ranaviruses, although there is new information that GV–6 is more distinct from SCRV than previously considered (Holopainen et al. 2009). GV–6 is thus considered unlikely to establish or spread in foodfish or free-living susceptible host populations in Australia.

- Challenge trials conducted in Europe show that ornamental fish of the families Cichlidae, Poeciliidae and the subfamily Luciocephalinae may be carriers of piscine ranaviruses (for example, ESV/ECV). Susceptible host species in natural waters may be exposed to ESV/ECV via ornamental fish entering natural waters via deliberate or accidental release, or inadvertent escape from ground level ponds during flooding. ESV/ECV is known to infect fish of the family Silurinidae under natural conditions, although experimental susceptibility of pike (*Esox lucius*) (family Esocidae) and pike-perch (*Sander lucioperca*) (family Percidae) have been demonstrated in Europe (Ariel 2009). Introduced redfin perch (*Perca fluviatilis*) of the family Percidae are found free-living in the cooler parts of the Australian Capital Territory, New South Wales, Victoria, Tasmania, South Australia and south-western Western Australia and represents a species in which ESV and ECV could establish. However, experimental transmission studies have shown that the redfin perch in Europe are not susceptible to ESV/ECV, suggesting that redfin perch populations in Australia may differ from European counterparts in their susceptibility to ESV/ECV.

- RTRV has been shown to infect marble goby of the family Eleotridae. Related species are found in natural waters in Australia (for example, striped gudgeons (*Gobiomorphus australis*), and flathead gudgeons (*Philypnodon grandiceps*). They are found free-living in sub-tropical and tropical parts of Australia and represent species in which the agent could establish and spread. Gudgeons may be used as feeder fish in the aquarium industry—they are collected from the wild migrations or ponds where they have bred and fed to larger fish including exotics. A species of the same family, sleepy cod (*Oxyeleotris lineolata*), is farmed in Australia on an experimental scale.

Use of ornamental fish as food for farmed foodfish or bait:

- Ornamental fish species extensively bred in large numbers in Australia are more likely to be used routinely as feed for farmed foodfish broodstock and as bait for recreational fishing compared to less readily available species. Goldfish and poeciliids are produced in large numbers in Australia, with cichlids and gouramis representing a smaller but growing sector.
- There is anecdotal evidence of the use of goldfish as bait (D. Ogburn, New South Wales Department of Primary Industries, pers. comm. August 2005, T. Hawkesford, Queensland Department of Primary Industries, pers. comm. September 2005), as well as similar (albeit infrequent) use of guppies, loaches and other small fish (M. Deveney, PIRSA, pers. comm. August 2005). Use of ornamental cichlids or zebrafish as bait is not known or suspected to occur, although it can be speculated that pond cultured locally bred gouramis may also be used as bait by fishers if the cost of purchase is relatively cheap.

Susceptibility of fish and amphibians in Australia to iridoviruses:

- Susceptibility of native or introduced fish species in aquaculture or in natural waters to iridoviruses is another factor critical to the likelihood of disease spread. The susceptibility of native fish species is unknown with respect to iridoviruses of quarantine concern, other than the demonstrated susceptibility of Murray cod to DGIV through experimental studies. Generally, megalocytiviruses can infect and potentially cause disease in a wide range of host species. Thus, it is reasonable to assume that iridoviruses of quarantine concern may have a broad host range, which may include Murray cod and other native and introduced species.
- Several ornamental fish families have formed self-maintaining populations in Australia, including cichlids, cyprinids and poeciliids. Iridoviruses of quarantine concern can potentially establish or spread in these populations.
- Ranaviruses such as EHNV have been shown to be infective to Murray cod, mosquito fish (*Gambusia affinis*) and mountain galaxias (*Galaxias olidus*) in experimental studies (Langdon 1986), suggesting ranaviruses associated with fish may be capable of infecting a wide host range. GV–6 has been shown to be infective to rainbow trout and chinook salmon (*Oncorhynchus tshawytscha*) via bath immersion. In these same studies, pike (family Esocidae – fish from this family are not found in Australia) were found susceptible to clinical disease from a number of fish ranaviruses, including EHNV and ESV. Pike were also found to be a vector for ECV and FV–3 but these viruses were not pathogenic to pike. In natural outbreaks of ranaviral disease around the world, goldfish and *Gnathopogon* spp. (Cyprinidae), stickleback (Gasterosteidae) and marble goby (Eleotridae) have been shown to be infected with ranaviruses. These observations suggest that a number of farmed and wild fish species in Australia may be susceptible (for example, goldfish, carp and sleepy cod).
- Studies have shown that angelfish, pearl gourami and zebrafish are susceptible to GV–6 via bath exposure, suggesting that wild populations of cichlids and gouramis may be susceptible. Goldfish and carp were found not to be susceptible. Redfin perch were shown not to be susceptible to GV–6 by bath challenge at 15 °C and 25 °C (Bang Jensen 2009).

- There is no information on the infectivity of RTRV to fish species other than marble goby (family Eleotridae) and goldfish (family Cyprinidae). Related fish species belonging to families Eleotridae (for example, gudgeons) and Cyprinidae (goldfish and rosy barbs) are found in natural waters and species such as sleepy cod (family Eleotridae) are farmed in Australia on an experimental scale.
- Pike-perch (Family Percidae) can be a subclinical carrier of ESV and ECV, suggesting that redfin perch found in more temperate regions of Australia could be susceptible. However, experimental studies have shown that the redfin perch in Europe is not susceptible to ESV/ECV via bath exposure.
- Some native amphibians may be susceptible to amphibian ranaviruses carried by ornamental fish (for example, RTRV). Metamorphs and tadpoles of a native amphibian, the giant tree frog (*Litoria infrafrenata*) were shown to be susceptible to FV3-like viruses [e.g. Bufo marinus (cane toad) Venezuelan iridovirus] via subcutaneous injection (Hyatt et al. 1998). [Note that more than 16 species of *Litoria* spp. are threatened and five are critically endangered.] Further, there is evidence that fish cohabiting with amphibians in ponds could become infected with amphibian ranaviruses (RPV and Stickleback virus). Amphibians that become infected by cohabiting with ornamental fish may in turn spread the agent to susceptible fish or amphibian populations in natural waters.

Australian state/territory regulation and industry codes:

- Australian states and territories regulate commercial ornamental fish and foodfish aquaculture. Aquaculture operations are required to be licensed and approval must be obtained from regulatory agencies on various management practices, including water and waste disposal methods, but no translocation protocols aimed at managing fish health have been specifically developed for ornamental fish farms. Farms are also required to control fish escape, have approved disease control programs and report significant disease events. However, these regulations are mainly directed towards foodfish aquaculture and large scale ornamental fish breeders. There is no specific regulation of small scale ornamental fish breeders or hobbyists.
- Legislation in Australian states and territories, other than Western Australia, prohibits the release of live ornamental fish into natural waters including as fishing bait. Legitimate release of translocated fish can occur only with prior government approval. Despite these regulations, the practice still occurs, albeit rarely (T. Hawkesford, Queensland Department of Primary Industries, pers. comm. September 2005, K. Weaver, Department of Fisheries Victoria, pers. comm. October 2005). In addition, anglers who use live fish as bait are prone to discarding excess fish either into the waterways they are fishing, or into local dams and ponds to provide bait for subsequent fishing trips. These introductions provide the potential for disease spread to susceptible host populations in natural waters.
- The only government controls on the use of dead fish as bait are in Tasmania where the use of live or dead fish as bait is banned in all inland waters other than estuarine waters under the Inland Fisheries (Recreational Fishing) Regulations 1999 (Raadik 2001).
- The PIAA's National Code of Practice addresses issues such as proper disposal of dead aquatic animals and unwanted or sick fish. The PIAA code also encourages ornamental fish trade and industry practice in accordance with the state and territory regulations.

Industry practices in accordance with PIAA's code may not be widely practised as only about 25 per cent of the aquarium retailers are currently members of the PIAA.

- The report, Strategic approach to the management of ornamental fish in Australia (Natural Resource Management Ministerial Council 2006) recommends a national system be developed to regulate the aquarium industry and large scale hobby operators that are not covered by existing state and territory fisheries regulations. Effective implementation of such a system may reduce the risk of disease spread from the ornamental fish industry to Australian foodfish farms and fish in natural waters through a better stakeholder understanding of management and biosecurity issues. This system may also provide an avenue to monitor and control disease.
- Use of amphibians as bait is prohibited in many states and territories in Australia including Tasmania, New South Wales, Australian Capital Territory and Victoria. For instance frogs are protected under the *National Parks and Wildlife Act 1974* and it is illegal to use live or dead frogs as bait for fishing in New South Wales. In Victoria frogs' eggs, tadpoles and frogs—dead or alive—are protected wildlife under the *Wildlife Act 1975* and they are not allowed to be used as bait.
- There are no specific regulations or codes of practices to control the movement of amphibians into commercial aquaculture facilities, although measures to prevent fish escape and manage predators such as water rats and birds are documented in Queensland's Environmental Code of Best Practice for Freshwater Finfish Aquaculture(Donovan 1999).

Conclusions

Based on the considerations discussed here, the likelihood of establishment or spread for the identified outbreak scenario associated with local ornamental fish (exposure group 1) is estimated to be:

Megalocytiviruses	Cichlids, gouramis and poeciliids	–	moderate
Piscine ranaviruses	Cichlids, gouramis and poeciliids	GV–6 ESV/ECV	moderate moderate
	Zebrafish	GV–6 ESV/ECV	moderate moderate
Amphibian ranavirus	Goldfish	RTRV	moderate
Goldfish iridoviruses	Goldfish	GFV–1/2	very low

Likelihood of establishment or spread from exposure group 2—farmed foodfish

The 1999 IRA did not identify farmed freshwater foodfish as an exposure group and thus no likelihoods were estimated. The significance of this exposure group has since been identified in this assessment as a result of a detection of iridovirus in farmed Murray cod (Lancaster et al. 2003) and the transmission studies undertaken by Go and Whittington (2006). As such, considerations in this IRA on this exposure group constitute information not included in the 1999 IRA.

2010 IRA—new considerations

The department considers the following information relevant to a conclusion of the likelihood of establishment or spread associated with exposure of farmed foodfish:

Likelihood of agent establishment or spread in a local foodfish population

Environmental conditions under aquaculture (in relation to stocking densities, water quality etc) for farmed foodfish or ornamental fish are generally more conducive to agent transmission and therefore agent establishment, compared to conditions in natural waters. There are many reports of iridovirus infections in farmed fish populations, for example, ISKNV in Chinese perch (He et al. 2000), RTRV in marble goby (Prasankok et al. 2005), ESV in sheatfish (*Silurus glanis*) (Ahne et al. 1989) and ECV in catfish (*Ictalurus melas*) (Pozet et al. 1992).

Go and Whittington (2006) demonstrated the potential for virus from gouramis (characterised as a megalocytivirus) to infect and cause high mortality in Murray cod (a farmed native foodfish species) through cohabitation trials. The 1999 IRA considered the susceptibility of Australian native fish to freshwater iridoviruses of concern to be unknown, and that there was no evidence that any vulnerable or endangered species in Australia (including the Murray cod) would be affected. Mortalities in juvenile Murray cod in a Victorian aquaculture facility have been attributed to an iridovirus considered to be a minor variant of DGIV or ISKNV (genus *Megalocytivirus*).

Cichlid iridoviruses are yet to be characterised and their taxonomic relationship to megalocytiviruses is unclear. However, based on pathological lesions observed and the morphology of these viruses, it is reasonable to assume they are megalocytiviruses. Thus, cichlid iridoviruses may have the potential to infect and cause disease in a wide range of host species including Murray cod and other farmed native and introduced food species.

GV–6 has been shown to cause 5 per cent mortality in chinook salmon and 4 per cent mortality in rainbow trout via bath exposure in experimental trials. The family Salmonidae is well represented in Australia with farming of rainbow trout mostly in freshwater ponds and sea cages, and Atlantic salmon (*Salmo salar*) in sea cages. Hatcheries supplying sea cage based farms hold both broodstock and juvenile fish in freshwater. However, the likelihood of ornamental fish being used as feed in salmonoid hatcheries is considered to be negligible.

ESV/ECV is known to infect only fish of the family Siluridae under natural conditions. Silurids are neither farmed nor found free-living in Australia and thus the agent is unlikely to establish in foodfish farms or in natural waters. Although experimental studies conducted by Hedrick and McDowell (1995) showed that rainbow trout may be susceptible via bath exposure to ESV, subsequent studies suggest that European redfin perch (family Percidae) and rainbow trout (family Salmonidae) are not susceptible to ESV/ECV. In the same study, pike-perch (family Percidae) were shown to be susceptible to ESV.

The susceptibility of foodfish species farmed in Australia to goldfish iridoviruses is not known. No transmission studies have been undertaken with GFV–1 and GFV–2.

Likelihood of agent spread to other susceptible populations once established or spread in the exposed foodfish population

Release of foodfish and/or infectious waste into natural waters or other foodfish farms:

Water and waste discharge or the escape of fish from infected farms into natural waters represents a potentially significant pathway by which disease could spread to wild fish populations.

Hatcheries provide juveniles for on-growing in other areas and as such, represent a significant threat of disease spread to receiving grow-out operations.

Farmed native or introduced fish of commercial or conservation value may be introduced into natural waters to replenish depleted populations (New South Wales Department of Primary Industries 2005).

Australian state/territory regulation and industry codes:

- Most Australian states and territories regulate water and waste discharge. All aquaculture operations are required to be licensed and approval must be obtained from regulatory agencies on water disposal methods. Farms are also required to control fish escape, have approved disease control programs and report significant disease events. Some farmers abide by industry codes of practice. State/territory controls and industry codes would reduce the likelihood of disease spread from commercial foodfish aquaculture sites. However, the level of risk reduction depends on the enforcement of legislation by state and territory authorities and the level of compliance by aquaculturists. Some state and territories have field based officers that are available to take compliance action if a case arises.
- State and territory authorities generally look for good facility design and good biosecurity plans and procedures, before approving hatcheries.
- State/territory governments are usually responsible for stocking of natural waters and take precautions to ensure the health status of translocated fish (New South Wales Department of Primary Industries 2005). In Queensland, private hatcheries supply fingerlings if there are any restocking programs. These hatcheries are subject to general health standards and since 2002–03, have been subject to specific health testing and translocation protocols.

Conclusions

Based on the considerations above, the likelihood of establishment or spread associated with local farmed foodfish (exposure group 2) is determined to be:

Megalocytiviruses	Cichlids, gouramis and poeciliids	–	very low
Piscine ranaviruses	Cichlids, gouramis, poeciliids and zebrafish	GV–6 ESV/ECV	very low very low
Amphibian ranavirus	Goldfish	RTRV	very low
Goldfish iridoviruses	Goldfish	GFV–1/2	very low

Likelihood of establishment or spread from exposure group 3—susceptible host species in natural waters

1999 IRA—key considerations

The 1999 IRA took into account the following iridovirus-specific information in assessing the likelihood of pathogenic agent establishment or spread in susceptible fish in Australian natural waters:

- Cichlids and gouramis are susceptible to iridoviruses and are commonly traded in Australia.
- Horizontal transmission via ingestion of infected excreta and cannibalism of dead fish is likely.

2010 IRA—new considerations

In addition to the information presented in the 1999 IRA, the department considers the following relevant to a conclusion on the likelihood of establishment or spread for the identified outbreak scenario associated with the exposure of susceptible species in natural waters:

Likelihood of agent establishment or spread in susceptible host species in natural waters

- Establishment of a disease in any population, cultured or wild, depends on a number of factors relating to the host, pathogenic agent and the environment.
- In comparison with cultured fish, there are relatively few records of epizootics in the wild. However, this is not necessarily evidence of their absence or infrequency. Although, highly pathogenic organisms can cause high mortality when first introduced to naive populations, low level mortality/morbidity would be expected where a disease has established in a wild population. Such low level impacts could go unnoticed due to the inaccessible nature of the environment or rapid removal of dead or moribund fish through predation/scavenging. Subclinically infected fish are highly unlikely to be detected.
- Environmental conditions under aquaculture (in relation to stocking densities, water quality etc), whether for farmed foodfish or ornamental fish, are generally believed to be more conducive to agent transmission and therefore agent establishment, than is the case for transmission and establishment of disease in fish in natural waters. Relatively lower stress and densities of fish in natural waters make the likelihood of disease establishment less than that for fish under aquaculture; although the relative absence of stress and disease in wild fish may be overemphasised (Hedrick 1998). Examples of iridoviruses that have established in wild populations include SCRV in the United States (Plumb et al. 1999) and EHNV in Australia (Langdon 1986; Langdon and Humphrey 1987).
- The susceptibility of native and introduced fish species in aquaculture or in natural waters is unknown for megalocytiviruses, other than the susceptibility of Murray cod to DGIV. This assessment concludes that poeciliid and cichlid megalocytiviruses of quarantine concern may have a similar broad host range, which may include Murray cod and other native and introduced species.

- Populations of cichlids, goldfish, gouramis and poeciliids have formed self-maintaining populations in Australian natural waters (Arthington et al. 1999). These represent known populations in which iridoviruses of quarantine concern could become established.
- There is evidence that GV–6 is pathogenic to salmonids under experimental conditions. There are salmonid species (rainbow trout, brown trout) found free-living in Tasmania, Victoria and New South Wales. Guppies may be used as bait for trout fishing in streams and in stocked impoundments.
- Intake of natural waters by semi-closed hatcheries, such as that occurring in Tasmania may result in infected water entering hatcheries.
- ESV/ECV may establish in redfin perch and rainbow trout found in more temperate regions of Australia. However, studies have shown that redfin perch and rainbow trout in Europe are not susceptible to ESV/ECV via bath exposure, suggesting that redfin perch populations in Australia may differ from Europeans counterparts in their susceptibility to ESV/ECV.
- Reports of goldfish iridoviruses are restricted to a single publication on the isolation from healthy fish of GFV–1 and GFV–2 in primary cell culture. Given that there is only one report of this virus in a species that is extensively researched, this should be regarded as an incidental discovery.

Likelihood of agent spread to other susceptible populations once established or spread in the exposed population in natural waters

- It is very difficult (if not impossible) to eradicate aquatic animal pathogenic agents once they have established in natural waters. Thus, it is considered inevitable that once established in a fish population in natural waters, a pathogenic agent would eventually spread to other susceptible fish populations. Note that once amphibian ranavirus enters natural waterways it could infect free-living fish and amphibians.
- Aquaculture broodstock may sometimes be sourced from the wild. If an iridovirus of quarantine concern were present in such fish, it is unlikely that the agent would be detected. These fish represent a potential pathway for a pathogenic agent to spread from natural populations into susceptible populations of farmed foodfish and ornamental fish, particularly in less stringently regulated small scale or backyard operations. Although not very effective in detecting subclinically infected animals, most commercial hatcheries have in place disease prevention controls that include observation of newly introduced broodstock held under quarantine for 30 days, which would assist in detecting animals exhibiting clinical signs of disease.
- An iridovirus, thought to be a minor variant of DGIV or ISKNV, was detected in association with mortalities in farmed Murray cod. It is not known how the virus was introduced to the farm. It was speculated that it could have been through infected fingerlings from another hatchery that may have been infected through broodstock sourced from the wild (Lancaster et al. 2003).
- Feral ornamental fish populations could reasonably be expected to amplify megalocytiviruses and ranaviruses released to natural waters, thereby increasing the likelihood of exposure of other fish in those waters, native or introduced. For example, feral goldfish are widespread in farm dams throughout Tasmania, a situation likely to be

due to dumping of unwanted pets (Tasmanian Government response to the draft IRA report, 9 June 2009). The presence of goldfish in farm dams presents a pathway for further spread of iridoviruses into the natural waters from dam overflow carrying infected fish or contaminated water into natural waters.

- Goldfish are commonly reared in outdoor ponds and if not biosecure, wild amphibians may move in and out of these ponds freely resulting in the transmission of amphibian ranaviruses to other fish and amphibians.

Conclusions

Based on the considerations above, the likelihood of establishment or spread associated with susceptible host species in natural waters (exposure group 3) is determined to be:

Megalocytiviruses	Cichlids, gouramis and poeciliids	–	low
Piscine ranaviruses	Cichlids, gouramis, poeciliids and zebrafish	GV–6 ESV/ECV	very low very low
Amphibian ranavirus	Goldfish	RTRV	very low
Goldfish iridoviruses	Goldfish	GFV–1/2	extremely low

5.3.2 Impacts

This section describes the evaluation of the biological, economic and environmental impacts associated with the scenario in which the agent establishes in exposed populations and spreads to other natural and farmed populations of susceptible species in Australia.

1999 IRA—key considerations

The 1999 IRA identified a number of general points in relation to establishment or spread. These included:

- epidemiological differences between diseases of aquatic and terrestrial animals
- the economic significance of diseases caused by opportunistic pathogenic agents when environmental conditions are favourable
- the complex interaction between host, environmental (including husbandry in fish facilities) and agent factors.

More specific points were also identified and these included:

- Australia's capacity to respond to fish disease incursions
- the economic effects associated with establishment or spread
- the zoning or regionalisation of disease if an exotic disease were to become established, to limit its further spread
- effects on industry due to outbreaks of disease in wholesale or retail facilities, commercial or breeding premises and hobbyist aquariums
- ecological and environmental effects such as the survival of native species (including amphibians), a decline in the numbers of endangered or threatened species and the extinction of a species.

For iridoviruses of quarantine concern associated with freshwater ornamental fish, the 1999 IRA determined the consequence to native and introduced species, both wild and captive, to be 'negligible' to 'low'.

Impacts due to establishment or spread of iridovirus

1999 IRA—key considerations

The 1999 IRA took into account the following iridovirus specific information in assessing impacts associated with agent establishment or spread in the ornamental fish industry:

- Ram cichlids (*Mikrogeophagus ramirezi*) appear to be highly susceptible, experiencing 100 per cent morbidity and 40 to 80 per cent mortality (Leibovitz and Riis 1980b).
- Anderson et al. (1993) reported iridovirus-like virions by electron microscopy in the stromal cells of the lamina propria in dwarf gourami. However, virus-associated lesions were not observed, suggesting that viral infection did not contribute to the mortality of fish.
- Iridovirus was isolated from two separate stocks of diseased three-spot gouramis (Fraser et al. 1993). The virus was isolated from the spleen and intestines of moribund fish, although aetiology of the disease was not confirmed. However, mortalities reached 70 per cent with lesions of a type that indicated systemic iridovirus infection was the cause of death.
- Rodger et al. (1997) reported systemic iridovirus infection in freshwater angelfish, with mortalities higher than 70 per cent.
- Cichlids and gouramis are produced locally in Australia on a very small scale. Effects on industry of these iridoviruses establishing in freshwater ornamental fish are expected to be at the individual producer level and not at a whole industry level.
- The 1999 IRA took into account the following iridovirus-specific information in assessing impacts associated with agent establishment in Australian natural waters, specifically ecological and environmental effects:
  - The susceptibility of Australian native fish to iridoviruses associated with freshwater ornamental fish is not known. There is experimental evidence that cross-infection from frogs to fish can occur (Moody and Owens 1994). The reverse may also be possible but has not been shown.
  - The origin of BIV in Australia is not known. Although it may be possible that BIV originated from a fish iridovirus, a survey of literature did not reveal any information supporting a causal link between any ornamental fish related iridoviruses and BIV and reported declines in populations of native frogs.
  - There is currently no evidence that iridoviruses carried by freshwater ornamental fish would affect survival of any vulnerable or endangered species in Australia or have any significant effect on the environment.

For iridoviruses of quarantine concern associated with freshwater ornamental fish, the 1999 IRA determined the consequence to native and introduced species, both wild and captive, to be 'negligible' to 'low'.

2010 IRA—new considerations

In addition to information presented in the 1999 IRA, the department considers the following relevant to a conclusion on the economic, biological and environmental impacts of agent establishment or spread:

Direct criterion 1

Direct impacts on the life or health (including production effects) of fish within the ornamental fish industry and farmed foodfish (native and introduced fish)

Ornamental fish industry:

- In 2002–03 more than 7.2 million ornamental fish were reported to have been produced in Australia, valued at almost $5.0 million (farm-gate value). The 'backyard' ornamental fish breeding sector would not be included in these production figures. Northern Territory production is negligible and there is only a single farm producing ornamental fish in Tasmania (J. Patrick, Pet Industry Association of Australia, pers. comm. August 2005). A significant proportion of both imported and locally produced ornamental fish are cichlids, goldfish, gouramis and poeciliids.
- Megalocytiviruses have a wide host range and it is expected that cichlids, poeciliids and gouramis will be susceptible.
- Commercial and backyard gourami and cichlid breeders may be significantly affected by the establishment of megalocytiviruses in cichlids and gouramis in their facilities.
- Megalocytiviruses have been associated with significant disease in the poeciliids: green swordtails (*Xiphophorus hellerii*), southern platyfish (*Xiphophorus maculatus*–commonly referred to as platys) and sailfin mollies (*Poecilia latipinna*).
- GV–6 has not been shown to be pathogenic to poeciliids. Experimental studies in Europe have shown that gouramis, cichlids and zebrafish can be carriers. GV–6 is considered unlikely to cause significant disease in the ornamental fish industry.
- Goldfish infected with RTRV have shown mortality under natural conditions suggesting that there may be mortalities in goldfish and other related cyprinids in aquaria and ponds.
- Goldfish iridoviruses have been isolated only from goldfish and there have been no reports of disease in goldfish or any other species.

Farmed foodfish:

- Freshwater foodfish aquaculture is a significant commercial industry in Australia. In 2001–02 New South Wales produced 8.2 tonnes of Murray cod with a farm-gate value of $166 000 (ABARE 2006). In 2005–06 Australia produced 2 075 tonnes of barramundi valued at $17.2 million and 361 tonnes of silver perch valued at $3.3 million (no data are available for Murray cod) (ABARE 2007). In Queensland, the annual production of Murray cod, Mary river cod (*Maccullochella peelii mariensis*) and sleepy cod (*Oxyeleotris lineolata*) was valued at $113 000 (2005–06) (Lobegeiger and Wingfield 2007).
- The Atlantic salmon production sector covers both freshwater hatchery operations, and the on-growing of fish in marine farming operations. Tasmania is the largest producer of

Atlantic salmon but some production also occurs in Victoria and South Australia. Tasmania produced 19 219 tonnes of Atlantic salmon (excluding hatchery production) with a farm-gate value of $221 million in 2005–06 (ABARE 2007).

- Rainbow trout is one of the most common trout species grown in Australia. Victoria is the largest producer of trout followed by New South Wales and South Australia. Australian trout production (mostly rainbow trout) was reported to be 1,955 tonnes with a farm-gate value of $10.8 million in 2005–06 (ABARE 2007). Some trout farming also occurs in Western Australia.
- Native fish species commercially farmed in freshwater in Australia include barramundi, Murray cod, silver perch, Barcoo grunter, golden perch and sleepy cod.
- Freshwater fish farming in Australia is regionalised. Murray cod are farmed mainly in New South Wales. Barramundi are farmed in most states and territories and silver perch in New South Wales, Queensland and Western Australia (ABARE 2006). Barcoo grunter and golden perch are farmed in New South Wales, Victoria and Queensland. Sleepy cod are farmed in Queensland.
- Lancaster et al. (2003) reported the isolation of an iridovirus in association with farmed Murray cod experiencing significant mortalities. Murray cod susceptibility to clinical disease caused by megalocytivirus from gouramis has been demonstrated. Iridoviruses associated with gouramis would have significant impact on the farming of Murray cod or related species.
- Significant impacts would be expected from the establishment of gourami iridoviruses in the Murray cod farming industry. The potential impacts of other iridoviruses such as cichlid iridoviruses are not known, although the findings of Go and Whittington (2006) show that the level of host specificity is likely to be broader than that assumed in the 1999 IRA.
- There is evidence that SCRV is closely related but distinct from GV–6 (Holopainen et al. 2009). SCRV can infect multiple species, but is only known to cause mortality only in largemouth bass and Florida bass of the family Centrarchidae under natural conditions (Goldberg et al. 2003). Centrarchids are restricted to North America, with no closely related species in Australia.
- Hedrick and McDowell (1995) showed that GV–6 is pathogenic to rainbow trout and chinook salmon via bath exposure. Although the dead fish contained up to 10^8 $TCID_{50}$/g of tissue and had significant haematopoietic and hepatocellular necrosis, mortality was very low with only 4 per cent in rainbow trout and 5 per cent in chinook salmon. Impacts due to establishment or spread of GV–6 are expected to be discernible in farmed (produced at the Snobs Creek Hatchery – now operated under commercial lease – and several commercial fish farms and stocked into selected waters for recreational fishing) and free-living rainbow trout populations and any free living chinook salmon populations (found in two lakes in south-western Victoria).
- ESV/ECV impacts are only expected to be discernible in free living redfin perch and free living and farmed rainbow trout populations.

Conclusions

Based on the considerations above, the impacts of the establishment or spread of iridoviruses in Australia for direct criterion 1 (the direct impacts on the life or health (including production effects) of farmed native and introduced fish, and fish within the ornamental fish industry) is estimated to be:

Megalocytiviruses	–	minor at the regional level (impact score C in Table 4)
Piscine ranaviruses	GV–6 ESV/ECV	minor at the regional level (impact score C in Table 4)
Amphibian ranavirus	RTRV	minor at the regional level (impact score C in Table 4)
Goldfish iridoviruses	GFV–1/2	minor at the regional level (impact score C in Table 4)

Direct criterion 2

The direct impact on the environment, including the life and health of fish in natural waters, and any impacts on the non-living environment

- It is reasonable to assume that poeciliid and cichlid megalocytiviruses would have an effect on a range of native fish species (including Murray cod) similar to that of gourami iridoviruses. However, there is no evidence on the susceptibility of any fish species other than goldfish to goldfish iridoviruses.
- Kearney and Kildea (2001) state the following on the economic value of Murray cod as a recreational fishing target:

  'It is not possible at present to estimate the true economic significance of Murray cod but as the apex predator in the aquatic ecosystem (and an umbrella species), a key indicator of the well-being of the total ecosystem of the Murray-Darling Basin, the pinnacle target for recreational fishers, the highest priced commercial fish, the general community icon (flagship) species and the only freshwater fish known by most Australians, the real economic (and political) significance would be enormous'.
- A survey of literature did not reveal any new information (to that stated in the 1999 IRA) supporting a causal link between any ornamental fish-related ranaviruses (EHNV, ESV/ECV and GV–6) and reported declines in populations of native frogs. Thus, piscine ranaviruses are not expected to have any discernible impact on amphibian populations.
- FV3-like viruses, for example Bufo marinus (cane toad) Venezuelan iridovirus has the capacity to infect Australian native amphibians, and the susceptibility of the giant tree frog metamorphs and tadpoles has been demonstrated via subcutaneous injection (Hyatt et al. 1998). This finding suggests that viruses in the FV–3 group may impact on native populations of amphibians in Australia. Currently, more than 15 species of frogs of the families Hyalidae and Myobatrachidae are listed as critically endangered by the Department of the Environment. Eighteen frog species of the same families are listed as endangered and 12 as vulnerable. Thus, amphibian ranavirus RTRV is expected to have some impact on native species of frogs and are likely to cause losses in these species.
- FV3-like viruses are also capable of infecting tortoises (see Table 10) (Class Reptilia; Order Testudines).The western swamp tortoise (*Pseudemydura umbrina*) is classified as critically endangered, the gulf snapping turtle (*Elseya lavarackorum*) and the Mary river turtle (*Elusor macrurus*) are listed as endangered and the Bell's turtle (*Elseya belli*), the Bellinger river emydura (*Emydura macquarii signata*) and the Fitzroy river turtle (*Rheodytes leukops*) are listed as vulnerable under the EPBC Act 1999. All these

testudines are of the family Chelidae. FV3-like infections have been reported from tortoises overseas of the families Testudinidae, Emylidae and Trionychidae, but have not been reported from Australian testudines. There is no evidence to suggest that Australian species are susceptible to FV3-like viruses, although BIV has been shown to be infective to Australian tortoises under experimental conditions.

Conclusions

Based on the considerations above, the impacts of the establishment or spread of iridoviruses in Australia for direct criterion 2 (the direct impact on the environment, including the life and health of fish in natural waters, and any impacts on the non-living environment) are estimated to be:

Megalocytiviruses	–	minor at the national level (impact score E in Table 4)
Piscine ranaviruses	GV–6	minor at state and territory level (impact score D in Table 4)
	ESV/ECV	minor at state and territory level (impact score D in Table 4)
Amphibian ranavirus	RTRV	minor at the national level (impact score E in Table 4)
Goldfish iridoviruses	GFV–1/2	unlikely to be discernable at all levels (impact score A in Table 4)

Indirect criterion 1

Indirect impacts of new or modified eradication, control, surveillance or monitoring and compensation strategies or programs

- Since the adoption of AQUAPLAN, Australia has made significant progress on its preparedness and responses to aquatic disease emergencies. There are currently well advanced contingency plans for specific diseases of fish and Australia is better prepared to deal with the entry, establishment or spread of aquatic animal diseases. [AQUAPLAN is Australia's National Strategic Plan for Aquatic Animal Health. It is a broad comprehensive strategy to build and enhance capacity for the management of aquatic animal health in Australia. The plan outlines objectives and projects to develop a national approach to emergency preparedness and response, and to the overall management of aquatic animal health.]
- None of the megalocytiviruses of quarantine concern is listed by the OIE or listed by an Australian state or territory government as 'notifiable'. They are also not included in 'Australia's National List of Reportable Diseases of Aquatic Animals'. However, in the event of significant mortality of farmed foodfish or fish in natural waters, some costs associated might be incurred through a government response (for example, disease investigation, diagnosis and control measures, as demonstrated during the Murray cod incident in Victoria), although such response may be less likely with pathogenic agents not specifically listed as notifiable by any state or territory government.
- Amphibian ranaviruses are currently listed by the OIE and listed regionally by the OIE/NACA. They are also included in 'Australia's National List of Reportable Diseases of Aquatic Animals'. If an exotic strain of amphibian ranavirus enters Australia and results in a significant mortality event in wild amphibian populations, a government response may be mounted depending on the circumstances, with associated costs (for example, disease investigation, diagnosis and control measures).
- ESV/ECV is currently listed regionally by the OIE and is notifiable in a number of states and territories in Australia. These agents may establish in wild and farmed fish and may trigger a government emergency response.

Conclusions

Based on the considerations above, the impacts of the establishment or spread of iridoviruses in Australia for indirect criterion 1 (the indirect impacts of new or modified eradication, control, surveillance or monitoring and compensation strategies or programs) are estimated to be as follows:

Megalocytiviruses	–	minor at the state and territory level (impact score D in Table 4)
Piscine ranaviruses	GV–6 ESV/ECV	unlikely to be discernable at all levels (impact score A in Table 4) minor at state and territory level (impact score D in Table 4)
Amphibian ranavirus	RTRV	minor at the state and territory level (impact score D in Table 4)
Goldfish iridoviruses	GFV–1/2	unlikely to be discernable at all levels (impact score A in Table 4)

Indirect criterion 2

Indirect impacts on domestic trade or industry, including changes in consumer demand and impacts on other industries supplying inputs to, or utilising outputs from, directly affected industries

- In the case of an outbreak, interstate barriers to the trade of live ornamental fish would be unlikely.
- There may be some interstate barriers to the trade of live foodfish intended for human consumption.
- As the state/territory governments are usually responsible for stocking of natural waters and take precautions to ensure the health status of translocated fish, the occurrence of an exotic disease may impact on the trade of fish for translocation between states and territories.
    - If FV3-like viruses establish in amphibian populations in Australia, there may be some interstate barriers to trade of amphibians, but these impacts are unlikely to be discernible at any level.

Conclusions

Based on the considerations above, the impacts of the establishment or spread of iridoviruses in Australia for indirect criterion 2 (the indirect impacts on domestic trade or industry, including changes in consumer demand and impacts on other industries supplying inputs to, or utilising outputs from, directly affected industries) are estimated to be as follows:

Megalocytiviruses	–	minor at the regional level (impact score C in Table 4)
Piscine ranaviruses	GV–6 ESV/ECV	unlikely to be discernable at all levels (impact score A in Table 4) unlikely to be discernable at all levels
Amphibian ranavirus	RTRV	unlikely to be discernable at all levels
Goldfish iridoviruses	GFV–1/2	unlikely to be discernable at all levels (impact score A in Table 4)

Indirect criterion 3

Indirect impact on international trade, including loss of markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand

- The detection of or an outbreak of disease due to megalocytiviruses of quarantine concern in this IRA, in ornamental fish or foodfish is unlikely to affect international trade as they are not listed as notifiable by the OIE.
- An outbreak of ranavirus is unlikely to have a discernible effect on international trade.

Conclusions

Based on the considerations above, the impacts of the establishment or spread of iridoviruses in Australia for indirect criterion 3 (the indirect impact on international trade, including loss of markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand) are estimated to be as follows:

Megalocytiviruses	–	unlikely to be discernable at all levels (impact score A in Table 4, section 2.4.4)
Piscine ranaviruses	GV–6	unlikely to be discernable at all levels
	ESV/ECV	unlikely to be discernable at all levels
Amphibian ranavirus	RTRV	unlikely to be discernable at all levels
Goldfish iridoviruses	GFV–1/2	unlikely to be discernable at all levels

Indirect criterion 4

Indirect impact on the environment including biodiversity, endangered species and integrity of ecosystems

- There is evidence that Murray cod is susceptible to gourami iridovirus and can show high mortality.
- Significant impacts would be expected from the establishment of gourami iridoviruses in Murray cod populations. The impact of other iridoviruses of quarantine concern on Murray cod and other native fish species is not known. Many are listed as endangered or vulnerable under National, state and territory legislation (for example, Macquarie perch, Murray cod, silver perch and trout cod). However, the findings of Go and Whittington (2006) show that the level of host specificity is likely to be broader than that assumed in the 1999 IRA.
- Mortalities of native fish species may significantly affect the biodiversity of local ecosystems. Populations of threatened species such as Murray cod may be significantly affected by exotic disease, adding to the potential for extinction and associated loss of biodiversity and integrity of ecosystems. Murray cod is listed as a threatened species (classified as vulnerable) under the EPBC Act 1999 and listed as threatened under Victorian threatened species legislation and classified as vulnerable for management purposes.
- Establishment of GV–6 may have some impact on free-living rainbow trout and chinook salmon populations although it is unlikely to cause any adverse impact on the environment.
- Establishment of FV–3 group viruses can impact on the amphibian and native fish populations and may alter biodiversity of the local systems. A number of Australian amphibians are critically endangered or endangered.

Conclusions

Based on the considerations above, the impacts of the establishment or spread of iridoviruses in Australia for indirect criterion 4 (the indirect impact on the environment including biodiversity, endangered species and integrity of ecosystems) are estimated to be as follows:

Megalocytiviruses	–	minor at the national level (impact score E in Table 4, section 2.4.4)
Piscine ranaviruses	GV–6	unlikely to be discernable at all levels (impact score A in Table 4, section 2.4.4)
	ESV/ECV	unlikely to be discernable at all levels
Amphibian ranavirus	RTRV	minor at the national level
Goldfish iridoviruses	GFV–1/2	unlikely to be discernable at all levels

Indirect criterion 5

Indirect impact on communities, including reduced tourism, reduced rural and regional economic viability, the loss of social amenity and any 'side impacts' of control measures

- Tourism associated with recreational fishing can contribute significantly to rural and regional economies. Therefore, the impact on rural and regional economies could be significant where recreational fishing targets iconic native fish such as Murray cod, barramundi and free-living rainbow trout that are susceptible to iridoviruses. The impact may be short term for abundant native species; however, for threatened species the impact may be long term or permanent.
- Impacts on social amenity may be expected through impacts on recreational fishing. For Australian native fish species that are not listed as being threatened or vulnerable, the impact is likely to be short term (for example, impact on recreational fisheries after fish kills as have been demonstrated by the impact of SCRV on North American recreational bass fisheries). However, if iridoviruses were to establish or spread in threatened Australian native species then the impacts on recreational fishing may be longer term or permanent.
- There may be adverse impacts on the social or group benefits (examples of social benefits include companionship, competition, family cohesion, and time spent with friends, family and others) of recreational fishing because of reduction in numbers of fish available for fishing due to fish kills.
  - Declining amphibian populations can lead to loss of social amenity (such as loss of tourism).

Conclusions

Based on the considerations above, the impacts of the establishment or spread of iridoviruses in Australia for indirect criterion 5 (the indirect impact on the environment including biodiversity, endangered species and integrity of ecosystems) is estimated to be as follows:

Megalocytiviruses	–	minor at the state and territory level (impact score D in Table 4, section 2.4.4)
Piscine ranaviruses	GV–6	minor at the state and territory level
	ESV/ECV	minor at the state and territory level
Amphibian ranavirus	RTRV	minor at the state and territory level
Goldfish iridoviruses	GFV–1/2	unlikely to be discernable at all levels (impact score A in Table 4, section 2.4.4)

Estimates of the impact scores for each direct and indirect criterion and the overall impacts of the establishment or spread (outbreak) scenario for iridoviruses of concern are shown in Table 16.

Table 16 Impact scores for the establishment or spread of iridoviruses associated with cichlids, goldfish, gouramis (subfamilies Luciocephalinae and Macropodinae of the family Osphronemidae) and poeciliids

Direct/indirect effects	Megalocytiviruses			Piscine ranaviruses (GV–6)			Piscine ranaviruses (ESV/ECV)			Goldfish ranaviruses (RTRV)			Goldfish iridoviruses (GFV–1 and 2)		
	Level of impact	Magnitude of impact	Impact score	Level of impact	Magnitude of impact	Impact Score	Level of impact	Magnitude of impact	Impact score	Level of impact	Magnitude of impact	Impact score	Level of impact	Magnitude of Impact	Impact Score
Direct effects															
Animal health (including production effects) of farmed native and introduced fish, and fish within the ornamental fish industry	Regional	MN	C	Regional	MN	C	Regional	MN	C	Regional	MN	C	Local	UD	A
The environment (native animals, and non living environment)	National	MN	E	State and territory (multiple regional)	MN	D	State and territory (multiple regional)	MN	D	National	MN	E	State and territory (multiple regional)	UD	A
Indirect effects															
Costs associated with eradication, control, surveillance and monitoring and compensation	State and territory	MN	D	Local	UD	A	State and territory (multiple regional)	MN	D	State and territory (multiple regional)	MN	D	Local	UD	A
Domestic trade effects and impact on other associated industries	Regional	MN	C	Local	UD	A	Local	UD	A	Local	UD	A	Local	UD	A
International trade effects	Local	UD	A	Local	UD	A	Local	UD	A	Local	UD	A	Local	UD	A
Effects on biodiversity, endangered species and the integrity of ecosystems	National	MN	E	Local	UD	A	Local	UD	A	National	MN	E	Local	UD	A
Changes in tourism, side effects from control measures, and loss of social amenity	State and territory	MN	D	State and territory (multiple regional)	MN	D	State and territory	MN	D	State and territory	MN	D	Local	UD	A
Overall impact	–	–	MD	–	–	Low	–	–	Low	–	–	MD	–	–	N

UD Unlikely to be discernible. **MD** Moderate. **MN** Minor. **N** Negligible. Other letters refer to Table 4.

Overall impacts

Based on the impact scores for direct and indirect criteria in Table 16 and using the rules for combining direct and indirect impacts (section 2.4.4), the overall impacts of establishment or spread of megalocytiviruses in ornamental fish, farmed foodfish and fish in natural waters was assessed to be *moderate* (Table 16).

The overall impact of establishment or spread of ranaviruses in ornamental fish, farmed foodfish and fish in natural waters was assessed to be *low* (Table 16).

The overall impact of establishment or spread of uncharacterised iridoviruses in ornamental fish, farmed foodfish and fish in natural waters was assessed to be *negligible* (Table 16).

5.3.3 Determination of likely consequences

Having obtained an estimate of overall impacts associated with the identified scenario for each exposure group (Table 16), this was combined with the likelihood that the identified scenario would occur (from the conclusions determined in Chapter 5.3.1) using the combination rules in Table 5 for each outbreak scenario. Thus a scenario specific estimate of likely consequences was derived for each exposure group (Table 17).

Table 17 Estimation of likely consequences for each exposure group

Exposure Group		Megalocytiviruses	Piscine ranaviruses		Goldfish ranaviruses RTRV	Goldfish iridoviruses GFV–1 and 2
			GV– 6	ECV/ESV		
Ornamental fish industry	Likelihood of establishment or spread	Moderate	Moderate	Moderate	Moderate	Very low
	Overall impact	Moderate	Low	Low	Moderate	Negligible
	Likely consequences	Moderate	Low	Low	Moderate	Negligible
Farmed Foodfish	Likelihood of establishment or spread	Very low	Very low	Very low	Very low	Very low
	Overall impact	Moderate	Low	Low	Moderate	Negligible
	Likely consequences	Very Low	Negligible	Negligible	Very low	Negligible
Susceptible host species in natural waters	Likelihood of establishment or spread in natural waters	Low	Very low	Very low	Very low	Extremely low
	Overall impact	Moderate	Low	Low	Moderate	Negligible
	Likely consequences	Low	Negligible	Negligible	Very low	Negligible

5.4 Overall risk determination

5.4.1 Determination of the likelihood of entry and exposure

The likelihood of entry and exposure for each iridovirus of concern shown in Table 18 is determined by combining the likelihood of release from the conclusions in Chapter 5.1 with the likelihood of exposure from the conclusions in Chapter 5.2, using the rules for combining descriptive likelihoods in Table 3, section 2.3.

Table 18 Likelihood of entry and exposure for each iridovirus of concern

Pathogenic agent		Likelihood of release	Likelihood of exposure			Likelihood of entry and exposure		
			Group 1[a]	Group 2[b]	Group 3[c]	Group 1	Group 2	Group 3
Megalocytiviruses	Cichlid	High	Moderate	Very low	Very low	Moderate	Very low	Very low
	Gourami	High	Moderate	Very low	Very low	Moderate	Very low	Very low
	Poeciliid	High	Moderate	Very low	Low	Moderate	Very low	Low
Piscine ranaviruses GV-6 and ECV/ESV	Poeciliid	Very low	Moderate	Very low	Low	Very low	Extremely low	Very low
	Cichlid and gouramis	Very low	Moderate	Very low	Very low	Very low	Extremely low	Extremely low
	Zebrafish	Very low	Moderate	Very low	Negligible	Very low	Extremely low	Negligible
Amphibian ranaviruses	Goldfish	Very low	Moderate	Very low	Low	Very low	Extremely low	Very low
Goldfish iridoviruses	Goldfish	Very low	Moderate	Very low	Low	Very low	Extremely low	Very low

a Group 1 ornamental fish industry. **b** Group 2 farmed foodfish. **c** Group 3 fish in the natural environment.

5.4.2 Determination of the risk

The risk for each iridovirus of concern with respect to the relevant ornamental fish species shown in Table 19 is determined by combining the likelihood of entry and exposure from the conclusions in Table 18, section 5.4.1 with the likely consequences from Table 17, section 5.3.3, using the rules in the risk estimation matrix in Table 6, section 2.4.5.

Table 19 Exposure group specific risk for each iridovirus of concern

Pathogenic agent		Likelihood of entry and exposure			Likely consequences			Risk		
		Group 1[a]	Group 2[b]	Group 3[c]	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Megalocyti-viruses	Cichlid	Moderate	Very low	Very low	Moderate	Very low	Low	Moderate	Negligible	Negligible
	Gourami	Moderate	Very low	Very low	Moderate	Very low	Low	Moderate	Negligible	Negligible
	Poeciliid	Moderate	Very low	Low	Moderate	Very low	Low	Moderate	Negligible	Very low
Piscine ranaviruses – Poeciliids		Very low	Extremely low	Very low	Low	Negligible	Negligible	Negligible	Negligible	Negligible
Piscine ranaviruses – Cichlids and gouramis		Very low	Extremely low	Extremely low	Low	Negligible	Negligible	Negligible	Negligible	Negligible
Piscine ranaviruses – Zebrafish		Very low	Extremely low	Negligible	Low	Negligible	Negligible	Negligible	Negligible	Negligible
Goldfish ranaviruses		Very low	Extremely low	Very low	Moderate	Very low	Very low	Very low	Negligible	Negligible
Goldfish iridoviruses		Very low	Extremely low	Very low	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

a Group 1 ornamental fish industry. **b** Group 2 farmed foodfish. **c** Group 3 fish in the natural environment.

5.4.3 Conclusions

The overall risk (of release, exposure and establishment or spread) associated with each iridovirus group of quarantine concern (combining the three exposure group specific risks using the combination rules) and corresponding group of ornamental fish is shown in Table 20.

Table 20 Overall risk for each iridovirus of concern

Pathogenic agent	Host group	Overall risk
Megalocytiviruses	Cichlid, gourami and poeciliids	Moderate
Piscine ranaviruses: GV–6 and ESV/ECV	Poeciliids, cichlids, gouramis and zebrafish	Negligible
Amphibian ranavirus: RTRV	Goldfish	Very low
Goldfish iridoviruses: GFV–1 and 2	Goldfish	Negligible

Megalocytiviruses

As per the risk estimation matrix presented in Table 6 (section 2.4.5), overall risks determined to be low, moderate, high or extreme do not achieve Australia's ALOP (very low). As such, the risk posed by the importation of cichlids, gouramis (Subfamilies Luciocephalinae and Macropodinae of the family Osphronemidae) and poeciliids with respect to the release, exposure and establishment or spread of megalocytiviruses are moderate and require further risk management in addition to that currently in place.

Ranaviruses

The risk posed by the importation of poeciliids, cichlids, gouramis and zebrafish with respect to the release, exposure and establishment or spread of piscine ranaviruses are negligible and by the importation of goldfish with respect to the release, exposure and establishment or spread of goldfish ranavirus is very low. The overall risk associated with poeciliid, cichlid, gouramis, zebrafish piscine ranaviruses and goldfish amphibian ranaviruses therefore achieves Australia's ALOP without further risk management to that currently in place.

Goldfish iridoviruses

The risk posed by the importation of goldfish with respect to the release, exposure and establishment or spread of goldfish iridoviruses are negligible and therefore achieves Australia's ALOP without further risk management to that currently in place.

6 Risk management

The method for risk management described here is consistent with that described by the OIE, and is applied in turn to each of the pathogenic agents identified as posing an unrestricted risk that exceeds Australia's ALOP.

Because of the generic nature of this risk analysis, the department has based its evaluation on an assumption that the pathogenic agents of concern are present in the exporting country. Where exporting countries can provide specific data on their own disease status, including evidence to support disease freedom, the department will reconsider the risk assessment based on that data.

Note that reference to 'gouramis' in risk estimations in this chapter corresponds to fish of the subfamilies Luciocephalinae and Macropodinae of the family Osphronemidae.

For the following pathogenic agents, the unrestricted risk estimate was deemed not to achieve Australia's ALOP and as such, risk management measures would be necessary to reduce the risk associated with each to an acceptable level:

Pathogenic agent	Host group	Overall risk
Megalocytiviruses	Cichlids	Moderate
	Gouramis	Moderate
	Poeciliids	Moderate

The 'unrestricted risk' estimations took into account the risk management measures currently in place for freshwater ornamental fish. Accordingly, the iridovirus-specific risk management measures identified in section 6.1 would be in addition to the controls that currently apply to cichlids, gouramis and poeciliids.

6.1 Risk management options

Risk management options that could be applied in this instance are limited due to:

- the animals being live which rules out most quarantine treatments associated with non-viable product
- the demonstrated ability of gourami iridoviruses (and presumably other iridoviruses of quarantine concern) to infect fish without causing clinical signs of disease (asymptomatic carrier status), so that the detection of disease in fish in quarantine through visual observation is unreliable
- the unknown length of time that some fish can be asymptomatic carriers of gourami iridoviruses (and presumably other iridoviruses of quarantine concern), making it difficult to determine an appropriate quarantine period.

The following three potential risk management options that could be applied to live fish are considered in this report.

Quarantine detention

Specific information on incubation periods or carrier status of iridoviruses associated with freshwater ornamental fish is limited. Subclinically infected carriers may succumb to clinical infection during quarantine if they are stressed by transport. However, carrier fish that are

transported and held under good conditions may carry iridoviruses of quarantine concern without showing obvious clinical signs and are likely to be released from quarantine detention. Go and Whittington (2006) demonstrated the potential for gouramis to harbour iridovirus without exhibiting clinical signs of disease for at least 28 days. Current risk management measures for all cichlids and gouramis due to biosecurity risks associated with iridoviruses include a pre-export quarantine period of 14 days and post-arrival quarantine period of 14 days. Thus, quarantine detention is not considered adequate to reduce iridovirus-associated risk to an acceptable level. Consequently, the current 14-day post-arrival quarantine detention requirement (in addition to the pre-export quarantine period of 14 days) aimed at managing risks associated with iridovirus in gouramis and cichlids is unlikely to be effective.

Batch testing

Batch testing of susceptible species either prior to export under the supervision of an approved overseas competent authority or post-arrival while under quarantine control would reduce the likelihood of release sufficiently to reduce the overall risk to an acceptable level. Only those batches that test negative would be released. Testing of cichlids, gouramis and poeciliids for the megalocytiviruses would be required. Note that a batch (epidemiological unit) is defined by the OIE as 'a discrete population comprising a group of ornamental fish of a single species that share the same potential risk of exposure to a pathogen because they share a common aquatic environment or because management practices make it likely that a pathogen in one group of animals would quickly spread to other animals'.

Pre-export or post-arrival batch testing should be at a standard which provides 95 per cent confidence of detecting the agent if it is present at a prevalence of 5 per cent. The level of protection provided by testing would depend on the availability of effective tests (including with respect to their sensitivity and commercial availability, as well as sampling and other operational procedures).

The department considers molecular tests shown to be able to detect subclinically infected fish such as the tests described by Go et al. (2006) and Jeong et al. (2008b) as suitable for pre-export batch testing.

The OIE standard PCR test for Red sea bream iridovirus (RSIV) is not considered suitable at this point in time as there is insufficient evidence that the test is capable of detecting virus presence in subclinically infected ornamental fish. A real-time PCR test, considered to have the highest sensitivity of all known tests is commercially available through the University of Sydney.

Due to industry (through PIAA) preference for pre-export testing and the practical feasibility of post-arrival testing, the option of post-arrival testing will only be considered on a case-by-case basis under special circumstances.

As a means of monitoring the effectiveness of overseas systems that underpin attestations about pre-export batch testing, it is recommended that imported shipments of cichlids, gouramis and poeciliids are subject to an on-going program of random post-arrival testing for megalocytivirus.

For the purpose of pre-export batch testing, a batch is defined as fish of a single species sharing water in a single holding system at the time of sample collection and that have remained epidemiologically isolated for at least 14 days from fish not of equivalent health status prior to export.

Sourcing from free stocks

Importation of ornamental fish could be permitted from countries, zones or compartments determined to be free of megalocytiviruses based on active (targeted testing) surveillance. A compartment is defined in the OIE Aquatic Code (2009) as 'one or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purpose of international trade. Such compartments must be clearly documented by the Competent Authority(ies)'. Surveillance should be based on PCR tests described as being suitable in the previous section under 'batch testing'. Determination of agent freedom would need to be to a standard consistent with that recommended by the OIE for diseases listed by the OIE, or to an equivalent standard for those diseases not listed by the OIE. For Australian government authorities to be satisfied that a country, zone or compartment is free of a given disease, they must have a knowledge of the competent authority (for example, the veterinary services or equivalent) of that country and be satisfied that the competent authority has the capacity for disease control, monitoring and surveillance as appropriate for the disease. In some cases, it might be necessary for the disease to be subject to compulsory reporting or disease investigation.

An assessment of any application for approval of compartmentalisation or stock accreditation schemes would be undertaken to ensure that effective biosecurity measures are implemented and maintained throughout the complete chain from source population to point of export. A detailed submission would need to be provided by the competent authority of the exporting country and Australia would conduct an on-ground assessment of the proposed compartment or stock accreditation scheme.

Importation of at-risk fish species – cichlids, gouramis and poeciliids – from free countries, zones or compartments is expected to reduce the overall risk associated with megalocytiviruses so as to achieve Australia's ALOP, subject to a satisfactory assessment of the country's competent authority and its capacity to determine and maintain disease freedom.

As a means of monitoring the effectiveness of overseas systems that underpin attestations about country, zone or compartment freedom, it is recommended that imported shipments of cichlids, gouramis and poeciliids are subject to an on-going program of random post-arrival testing for megalocytivirus.

6.2 Pathogenic agent specific risk management measures

Megalocytiviruses

The overall unrestricted risks associated with cichlid, gourami and poeciliid megalocytiviruses were estimated as *moderate*. The department considers that the following risk management measures would each reduce the overall megalocytivirus risk from *moderate* to at least *very low*, thereby achieving Australia's ALOP:

- country, zone or compartment freedom OR
- pre-export batch testing.

Each of these measures would reduce the likelihood of release to at least *very low*.

The restricted risk determinations for country, zone or compartment freedom or pre-export batch testing are summarised in Table 21.

Table 21 Restricted risk estimations after pre-export batch testing for megalocytivirus or by sourcing from a megalocytivirus free country, zone or compartment

Risk element	Exposure group	Cichlid megalocytivirus		Gourami megalocytivirus		Poeciliid megalocytivirus	
		Unrestricted risk	Restricted risk	Unrestricted risk	Restricted risk	Unrestricted risk	Restricted risk
Likelihood of release		H	VL	H	VL	H	VL
Likelihood of exposure	Ornamental fish industry	M	M	M	M	M	M
	Farmed foodfish	VL	VL	VL	VL	VL	VL
	Fish in natural waters	VL	VL	VL	VL	L	L
Likelihood of entry and exposure	Ornamental fish industry	M	VL	M	VL	M	VL
	Farmed foodfish	VL	EL	VL	EL	VL	EL
	Fish in natural waters	VL	EL	VL	EL	L	VL
Likely consequences	Ornamental fish industry	M	M	M	M	M	M
	Farmed foodfish	VL	VL	VL	VL	VL	VL
	Fish in natural waters	L	L	L	L	L	L
Risk	Ornamental fish industry	M	VL	M	VL	M	VL
	Farmed foodfish	N	N	N	N	N	N
	Fish in natural waters	N	N	N	N	VL	N
Overall risk		M	VL	M	VL	M	VL

EL Extremely low. **H** High. **L** Low. **M** Moderate. **N** Negligible. **VL** Very low.

6.3 Conclusions and recommendations

To achieve Australia's ALOP with respect to megalocytiviruses, all imported cichlids, gouramis (subfamilies Luciocephalinae and Macropodinae of the family Osphronemidae) and poeciliids would need to be:

- batch tested prior to export to Australia under the supervision of an approved overseas competent authority and found to be negative for megalocytiviruses,

  OR

- sourced from a country, zone or compartment determined to the satisfaction of Australian government authorities to be free of megalocytiviruses (based on active surveillance).

It is considered that either of these two measures, in addition to other pre-export quarantine measures and relevant official health certification, would achieve Australia's ALOP with respect to iridovirus associated risks. The current 14-day post-arrival quarantine detention period aimed at managing iridovirus-specific risk is considered unlikely to provide any additional

assurance — a quarantine detention period of seven days would still apply as part of the baseline measures currently applied to all imported ornamental fish.

As a means of monitoring the effectiveness of overseas systems that underpin attestations about batch testing or country, zone or compartment freedom, it is recommended that imported shipments of cichlids, gouramis and poeciliids are subject to an on-going program of random post-arrival testing for megalocytivirus.

The diagnostic tests used (for example, PCR) for purposes of batch testing or demonstration of country, zone or compartment freedom must be appropriate for the purpose and adequately sensitive. The department considers PCR tests shown to be able to detect subclinically infected fish such as that described by Go et al. (2006) and Jeong et al. (2008b) as suitable for pre-export batch testing. Insufficient evidence that the OIE standard PCR test for RSIV is capable of detecting virus in subclinically infected ornamental fish is available. A real-time PCR test, considered to have the highest sensitivity of all known tests, is commercially available through the University of Sydney.

The department understands that this technology may be available to countries for demonstrating freedom for iridoviruses and for post-arrival batch testing, but that it will be a matter for the laboratory undertaking the testing to acquire the appropriate technology. Any surveillance sampling or testing must be consistent with general OIE international standards; in this case, the sampling/testing regime would be required to provide a 95 per cent confidence level of detecting the pathogenic agent if present at a prevalence of 5 per cent or more.

Equivalent approaches to managing identified risks may be accepted, either generally or on a case-by-case basis. Parties seeking to use alternative equivalent risk management measures to those identified would need to provide a submission for consideration. Such proposals should include supporting scientific data that clearly demonstrate equivalence of the proposed alternative measures.

7 Recommended quarantine measures for the importation of live freshwater ornamental fish with respect to iridoviruses

It is recommended that the following quarantine requirements apply to the importation of live freshwater ornamental fish (freshwater fish listed on Part 1 of the 'List of specimens taken to be suitable for live import'—'Live specimens that do not require an import permit' under the *Environmental Protection and Biodiversity Conservation Act 1999)* with respect to megalocytiviruses, and are consistent with the requirements of the *Quarantine Act 1908* and its subordinate legislation.

7.1 Import permit

The importer must obtain a permit from the Department of Agriculture to import all freshwater ornamental fish into Australia, before the goods are imported.

The application to import must include:

- the name and address of the importer and exporter; and
- a description of the commodity to be imported.

The application will be assessed on this information as well as any other criteria deemed relevant by Australia's Director of Animal and Plant Quarantine.

The department's import conditions apply to freshwater ornamental fish listed on the import permit at the time of importation.

Note: In assessing import permit applications, decision-makers must address the requirements in section 70 of the Quarantine Proclamation 1998 and consider the level of quarantine risk if the permit were granted and whether it is necessary to impose conditions to limit the level of risk to one that is acceptably low.

7.2 Live freshwater ornamental fish—poeciliids (family Poeciliidae), cichlids (family Cichlidae) and gouramis (subfamilies Luciocephalinae and Macropodinae of the family Osphronemidae)

7.2.1 All imported live poeciliids, cichlids and gouramis must be:

- batch tested prior to export (post-arrival batch testing will be considered on a case-by-case basis under special circumstances) under the supervision of an approved overseas competent authority and found to be negative for megalocytiviruses

  OR

- sourced from a country, zone or compartment that is recognised by Australia to be free of megalocytiviruses (based on active surveillance).

Tests shown to be capable of detecting subclinically infected fish such as those described by Go et al. (2006) and Jeong et al. (2008b) and a real-time PCR test, commercially available through the University of Sydney, are considered suitable for purposes of pre-export batch testing or active surveillance. Sampling must be at a level that provides 95 per cent confidence of detecting the agent if it is present at a prevalence of 5 per cent. Appendix E provides details on the numbers of fish that would need to be tested from each batch to provide 95 per cent confidence of detecting the agent if it is present at a prevalence of 5 per cent.

For the purpose of pre-export batch testing, a batch is defined as fish of a single species sharing water in a single holding system at the time of sample collection and that have remained epidemiologically isolated for at least 14 days from fish not of equivalent health status prior to export. Documentation from the competent authority of the exporting country identifying the batch and corresponding test results for the consignment must be provided to the department at the border prior to inspection.

7.2.2 For cichlids, gouramis and poeciliids, the Competent Authority in the exporting country must certify that:

- each batch of fish from which the fish in the consignment are derived has been tested using an approved test and found negative for megalocytivirus or has been sourced from a country, zone or compartment recognised by Australia to be free of megalocytiviruses (based on active surveillance).
- the fish in the consignment have been inspected within seven days prior to export and show no clinical signs of disease or pests.
- the export premises is currently approved for export to Australia by the Competent Authority.
- the fish being held at the export premises exhibit no signs of significant infectious disease or pests and are sourced from populations not associated with any significant disease or pests within the previous six months.
- the fish in the consignment have been in premises approved for the export of freshwater finfish to Australia for the 14 days prior to export.
- the fish have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing or koi carp).
- adequate safeguards are in place to maintain certified fish health status until export. Fish are effectively isolated in holding systems that prevent infection by direct contact with other fish or indirect contact via water, equipment or any other means.

7.2.3 All shipments of cichlids, gouramis and poeciliids will be subject to an on-going program of random post-arrival testing for megalocytivirus.

7.2.4 All shipments of fish will be inspected by Department of Agriculture officers on arrival to ensure they are healthy, are an approved species on the permitted species list and that they do not contain prohibited material or materials of quarantine concern. Any prohibited fish species will be exported or destroyed, while prohibited material and material of quarantine concern will be seized and destroyed at the importer's expense.

7.2.5 All fish will be ordered into quarantine detention on arrival in approved quarantine premises for a minimum seven days.

7.3 Review

Conditions for importation may be reviewed if there are any changes in the source country's import policy or its animal disease status, or at any time at the discretion of the Australia's Director of Animal and Plant Quarantine.

Appendix A: Changes to the final IRA report from the 2009 draft report

The following details the main changes in the final IRA report (this report) since the draft IRA report was released for stakeholder comment in March 2009. It includes changes as a result of stakeholder comments on the draft IRA report and new scientific information.

The draft IRA report considered risks associated with iridoviruses as a whole, including megalocytiviruses and ranaviruses as relevant to gouramis, cichlids, poeciliids and goldfish. In order to better address stakeholder comments on specific risks associated with ranaviruses and megalocytiviruses of poeciliids, and taking into consideration significant new information from a European Commission risk assessment of new and emerging systemic iridoviral diseases of European fish and aquatic ecosystems (the RANA project), the final IRA report considers the range of viruses of concern by grouping them, for purposes of risk assessment, as follows:

- Megalocytiviruses (ISKNV-like viruses) – in cichlids, gouramis and poeciliids
- Piscine ranaviruses – in poeciliids, cichlids, gouramis and zebrafish
- Amphibian ranaviruses – in goldfish
- Goldfish iridoviruses (GFV–1 and GFV–2) – in goldfish.

Using this approach, the Department of Agriculture has re-evaluated the risk associated with poeciliid ranavirus (GV–6) separately from poeciliid megalocytivirus and concluded that the risk associated with GV–6 meets Australia's ALOP and does not require additional risk management.

Studies undertaken as part of the European Commission's RANA Project also showed that a number of ornamental fish species are susceptible under experimental conditions to amphibian ranaviruses. The approach of grouping pathogens has enabled the department to better consider and differentiate risks associated with amphibian ranaviruses (RTRV) and piscine ranaviruses (ECV/ESV).

Furthermore, the role of amphibians in potential disease spread from ornamental fish ponds to other susceptible host species in the natural environment has been reviewed and given more prominence in this report. An amphibian technical information section, including new information, has been added to Chapter 2 (Technical Background). Appendix D in the draft IRA report on Amphibian iridoviruses has been removed and the information incorporated into Chapter 2.

For fish of the 'gourami family' (Osphronemidae), the draft IRA report recommended testing only for fish of the subfamily Luciocephalinae. Taking into consideration the finding of megalocytivirus in paradise fish after the release of the draft report, the final report recommends that megalocytivirus testing of the 'gourami family' be broadened to include fish of the subfamily Macropodinae, which includes Siamese fighting fish (bettas), paradise fish, licorice gouramis, pygmy gouramis and croaking gouramis.

The final IRA report also includes new information on the detection of megalocytivirus in post-arrival quarantine in Australia since release of the draft IRA report in March 2009. Several stakeholders questioned the final risk estimation for megalocytiviruses associated with poeciliids, as (at the time of the release of the draft IRA report) only one naturally occurring

disease outbreak had been reported. However, this outbreak involved a number of species and is further supported by the experimental findings of Jeong et al. (2008b). In addition, there has been a detection of a megalocytivirus from a poeciliid in post-arrival quarantine in Australia since release of the draft IRA report. The department has reviewed the risk assessment based on these findings and concluded that the risks associated with poeciliid megalocytivirus would not meet Australia's ALOP and would require risk management, similar to cichlids and gouramis.

The draft and provisional final IRA reports' recommended risk management options included post-arrival batch testing for megalocytivirus. Taking into consideration industry representations about the commercial feasibility of post-arrival batch testing, the final IRA report recommends batch testing prior to export under the supervision of an approved competent authority. Post-arrival batch testing will be considered on a case-by-case basis under special circumstances.

As announced in Biosecurity Advice 2012/01, the then Director of Animal and Plant Quarantine decided to await the completion of a University of Sydney survey of Australian fish for gourami iridovirus before making a determination on the proposed final IRA. The survey has been completed and its findings are considered consistent with the assumptions in the IRA. Although the department has monitored the scientific literature since the release of the draft provisional final IRA report, new scientific information has not been added to this report, since it has not been of a kind that would change the IRA's conclusions.

Appendix B: Biosecurity framework

Australia's biosecurity policies

The objective of Australia's biosecurity policies and risk management measures is the prevention or control of the entry, establishment or spread of pests and diseases that could cause significant harm to people, animals, plants and other aspects of the environment.

Australia has diverse native flora and fauna and a large agricultural sector, and is relatively free from the more significant pests and diseases present in other countries. Therefore, successive Australian Governments have maintained a conservative, but not a zero-risk, approach to the management of biosecurity risks. This approach is consistent with the World Trade Organization's (WTO's) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement).

The SPS Agreement defines the concept of an 'appropriate level of protection' (ALOP) as the level of protection deemed appropriate by a WTO Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory. Among a number of obligations, a WTO Member should take into account the objective of minimising negative trade effects in setting its ALOP.

Like many other countries, Australia expresses its ALOP in qualitative terms. Australia's ALOP, which reflects community expectations through Australian Government policy, is currently expressed as providing a high level of sanitary and phytosanitary protection, aimed at reducing risk to a very low level, but not to zero.

Consistent with the SPS Agreement, in conducting risk analyses Australia takes into account as relevant economic factors:

- the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease in the territory of Australia
- the costs of control or eradication of a pest or disease
- and the relative cost-effectiveness of alternative approaches to limiting risks.

Roles and responsibilities within Australia's quarantine system

Australia protects its human, animal and plant life or health through a comprehensive quarantine system that covers the quarantine continuum, from pre-border to border and post-border activities.

Pre-border, Australia participates in international standard-setting bodies, undertakes risk analyses, develops offshore quarantine arrangements where appropriate, and engages with our neighbours to counter the spread of exotic pests and diseases.

At the border, Australia screens vessels (including aircraft), people and goods entering the country to detect potential threats to Australian human, animal and plant health.

The Australian Government also undertakes targeted measures at the immediate post-border level within Australia. This includes national co-ordination of emergency responses to pest and disease incursions. The movement of goods of quarantine concern within Australia's border is

the responsibility of relevant state and territory authorities, which undertake inter- and intra-state quarantine operations that reflect regional differences in pest and disease status, as a part of their wider plant and animal health responsibilities.

Roles and responsibilities within the department

The Australian Government Department of Agriculture is responsible for the Australian Government's animal and plant biosecurity policy development and the establishment of risk management measures. The Secretary of the department is appointed as the Director of Animal and Plant Quarantine under the *Quarantine Act 1908* (the Act).

The department takes the lead in biosecurity and quarantine policy development and the establishment and implementation of risk management measures across the biosecurity continuum, and:

- Pre-border conducts risk analyses, including IRAs, and develops recommendations for biosecurity policy as well as providing quarantine policy advice to the Director of Animal and Plant Quarantine
- At the border develops operational procedures, makes a range of quarantine decisions under the Act (including import permit decisions under delegation from the Director of Animal and Plant Quarantine) and delivers quarantine services
- Post-border coordinates pest and disease preparedness, emergency responses and liaison on inter- and intra-state quarantine arrangements for the Australian Government, in conjunction with Australia's state and territory governments.

Roles and responsibilities of other government agencies

State and territory governments play a vital role in the quarantine continuum. The department works in partnership with state and territory governments to address regional differences in pest and disease status and risk within Australia, and develops appropriate sanitary and phytosanitary measures to account for those differences. Australia's partnership approach to quarantine is supported by a formal Memorandum of Understanding that provides for consultation between the Australian Government and the state and territory governments.

Depending on the nature of the good being imported or proposed for importation, the Department of Agriculture may consult other Australian Government authorities or agencies in developing its recommendations and providing advice.

As well as a Director of Animal and Plant Quarantine, the Act provides for a Director of Human Quarantine. The Australian Government Department of Health is responsible for human health aspects of quarantine and Australia's Chief Medical Officer within that Department holds the position of Director of Human Quarantine. The Department of Agriculture may, where appropriate, consult with that Department on relevant matters that may have implications for human health.

The Act also requires the Director of Animal and Plant Quarantine, before making certain decisions, to request advice from the Environment Minister and to take the advice into account when making those decisions. The Australian Government Department of the Environment is responsible under the *Environment Protection and Biodiversity Conservation Act 1999* for assessing the environmental impact associated with proposals to import live species. Anyone

proposing to import such material should contact the Department of the Environment directly for further information.

When undertaking risk analyses, the Department of Agriculture consults with the Department of the Environment about environmental issues and may use or refer to the Department of the Environment's assessment.

Australian quarantine legislation

The Australian quarantine system is supported by Commonwealth, state and territory quarantine laws. Under the Australian Constitution, the Commonwealth Government does not have exclusive power to make laws in relation to quarantine, and as a result, Commonwealth and state quarantine laws can co-exist.

Commonwealth quarantine laws are contained in the *Quarantine Act 1908* and subordinate legislation including the Quarantine Regulations 2000, the Quarantine Proclamation 1998, the Quarantine (Cocos Islands) Proclamation 2004 and the Quarantine (Christmas Island) Proclamation 2004.

The quarantine proclamations identify goods which cannot be imported, into Australia, the Cocos Islands and or Christmas Island unless the Director of Animal and Plant Quarantine or delegate grants an import permit or unless they comply with other conditions specified in the proclamations. Section 70 of the Quarantine Proclamation 1998, section 34 of the Quarantine (Cocos Islands) Proclamation 2004 and section 34 of the Quarantine (Christmas Island) Proclamation 2004 specify the things a Director of Animal and Plant Quarantine must take into account when deciding whether to grant a permit.

In particular, a Director of Animal and Plant Quarantine (or delegate):

- must consider the level of quarantine risk if the permit were granted, and
- must consider whether, if the permit were granted, the imposition of conditions would be necessary to limit the level of quarantine risk to one that is acceptably low, and
- for a permit to import a seed of a plant that was produced by genetic manipulation – must take into account any risk assessment prepared, and any decision made, in relation to the seed under the *Gene Technology Act*, and
- may take into account anything else that he or she knows is relevant.

The level of quarantine risk is defined in section 5D of the *Quarantine Act 1908*. The definition is as follows:

A reference in this Act to a level of quarantine risk is a reference to:

(a) the probability of:

(i) a disease or pest being introduced, established or spread in Australia, the Cocos Islands or Christmas Island, and

(ii) the disease or pest causing harm to human beings, animals, plants, other aspects of the environment, or economic activities, and

(b) the probable extent of the harm.

The Quarantine Regulations 2000 were amended in 2007 to regulate keys steps of the IRA process.

The Regulations:

- define both a standard and an expanded IRA
- identify certain steps which must be included in each type of IRA
- specify time limits for certain steps and overall timeframes for the completion of IRAs (up to 24 months for a standard IRA and up to 30 months for an expanded IRA);
- specify publication requirements
- make provision for termination of an IRA
- allow for a partially completed risk analysis to be completed as an IRA under the Regulations.

The Regulations are available at Comlaw.

International agreements and standards

The process set out in the Import risk analysis handbook 2011 is consistent with Australia's international obligations under the SPS Agreement. It also takes into account relevant international standards on risk assessment developed under the International Plant Protection Convention (IPPC) and by the World Organisation for Animal Health (OIE).

Australia bases its national risk management measures on international standards where they exist and when they achieve Australia's ALOP. Otherwise, Australia exercises its right under the SPS Agreement to apply science-based sanitary and phytosanitary measures that are not more trade restrictive than required to achieve Australia's ALOP.

Notification obligations

Under the transparency provisions of the SPS Agreement, WTO Members are required, among other things, to notify other members of proposed sanitary or phytosanitary regulations, or changes to existing regulations, that are not substantially the same as the content of an international standard and that may have a significant effect on trade of other WTO Members.

Risk analysis

Within Australia's quarantine framework, the Australian Government uses risk analyses to assist it in considering the level of quarantine risk that may be associated with the importation or proposed importation of animals, plants or other goods.

In conducting a risk analysis, the Department of Agriculture:

- identifies the pests and diseases of quarantine concern that may be carried by the good
- assesses the likelihood that an identified pest or disease or pest would enter, establish or
- spread, and
- assesses the probable extent of the harm that would result.

If the assessed level of quarantine risk exceeds Australia's ALOP, the department will consider whether there are any risk management measures that will reduce quarantine risk to achieve the ALOP. If there are no risk management measures that reduce the risk to that level, trade will not be allowed.

Risk analyses may be carried out by the department's specialists, but may also involve relevant experts from state and territory agencies, the Commonwealth Scientific and Industrial Research Organisation (CSIRO), universities and industry to access the technical expertise needed for a particular analysis.

Risk analyses are conducted across a spectrum of scientific complexity and available scientific information. An IRA is a type of risk analysis with key steps regulated under the Quarantine Regulations 2000. The department's assessment of risk may also take the form of a non-regulated analysis of existing policy or technical advice to the operational biosecurity areas of the department. Further information on the types of risk analysis is provided in the Import risk analysis handbook 2011.

Appendix C: Pet Industries Association of Australia (PIAA) special requirements for ornamental fish 2008

1. Retailers shall not trade in any fish or plant species listed as noxious or otherwise restricted in their state or territory.
2. In some states and territories there may be Government produced codes governing the operations of aquarium/aquatic outlets. These codes are enforceable.
3. Fish tanks must be protected from adverse environmental extremes.
4. Water changes must be adequate to maintain good water quality in relation to population density.
5. Unless other provisions are made, tank lids or other appropriate devices must be fitted and kept in place to prevent escape of fish.
6. Water chemistry must be checked regularly and appropriate measures taken to correct any imbalance.
7. All electrical equipment such as lights and heaters must be connected to safety switches and regularly checked for correct performance and safety.
8. Filtration equipment must be adequate for the species and tank/pond population densities, and effective at all times.
9. Fish must be fed as often as required with appropriate food according to species requirements.
10. All fish nets should be disinfected after use in each aquarium.
11. Fish showing signs of illness must be attended to immediately and where necessary, separated from other fish to prevent the spread of disease or molestation by healthy fish.
12. The use of medications in the treatment of diseased or injured aquatic animals shall be carried out quickly and humanely to provide a cure to the species concerned. Proper prescribed medications for the relevant disease must be used.
13. Any dead aquatic animal shall be disposed of in a manner that will not be the cause of a disease being released into natural waterways, for example, in garbage used as landfill, and not via storm water.
14. Retailers should suggest their customers advise them of unwanted aquatic animals and aquatic plants with a view to ‘re-homing’ them and preventing them being dumped into natural waterways. There is no obligation to repurchase, refund or take unquarantined animals into the shop/aquarium.
15. Retailers will advise and make relevant literature available to their customers to help educate them in responsible aquatic animal ownership.

Appendix D: Locations of ornamental fish established in Australian waters

Table D1 Summary of known locations of ornamental fish established in Australian waters in 2006 (Information based on Corfield et al. 2008)

Common name	Scientific name	Locations found in Australia
Family Cichlidae		
Hybrid cichlid	*Labeotropheus/ Pseudotropheus*	Hazelwood power station (Vic)
Jewel cichlid	*Hemichromis bimaculatus*	Rapid Creek in Darwin (NT); Ross River (northern Qld)
Victoria Burton's haplochromis	*Haplochromis burtoni*	Ross River in northern Qld and Hinze Dam (south-east Qld)
Black mangrove cichlid	*Tilapia mariae*	Cairns area, Barron, Ross, Johnstone, Burdekin, Mulgrave and Russel Rivers (Qld); Hazelwood power station, Eel Hole Creek., Latrobe River (Vic.); Lake Burley Griffin Canberra (ACT)
Redbelly tilapia	*Tilapia zillii*	Chapman River near Geraldton (WA)
Blue tilapia	*Oreochromis aureus*	No data obtained
Mozambique tilapia	*Oreochromis mossambicus*	Brisbane dams, Boyne River including Boondooma Dam, tidal Creeks around Townsville, Cairns, Atherton Tableland, Endeavour and Port Douglas; Barron, Ross, Mulgrave and North and South Johnstone and Pine Rivers, (Qld); Gascoyne, Lyons, Milnilya and Chapman Rivers in the Pilbara Drainage and limestone caves Exmouth (WA)
Oscar	*Astronotus ocellatus*	Ross River and creeks around Cairns (northern Qld)
Three-spot cichlid	*Cichlasoma trimaculatum*	Hinze Dam (south-east Qld)
Jack Dempsey	*Cichlasoma octofasciatum*	Angourie (northern NSW)
Firemouth cichlid	*Thorichthys meeki*	Ross River (northern Qld)
Banded cichlid	*Heros severus*	Ross River (northern Qld)
Redhead cichlid	*Vieja synspila*	No data obtained
Red devil	*Amphilophus labiatus*	Ross River (northern Qld); and Hinze Dam (south-east Qld); Hazelwood pondage, LaTrobe Valley (Vic)
Midas cichlid	*Amphilophus citrinellus*	Ross River (northern Qld)
Convict cichlid	*Archocentrus nigrofasciatus*	Ross River and streams around Townsville (northern Qld); Hazelwood power station, Eel Hole Creek, LaTrobe River (Vic.)
Blue acara	*Aequidens pulcher*	Creeks in Brisbane and Leslie Dam (south-east Qld); Hazelwood power station (Vic) Creeks in Brisbane and Leslie Dam (south-east Qld); Hazelwood power station (Vic)
Green terror	*Aequidens rivulatus*	Ross River (northern Qld)
Pearl cichlid	*Geophagus brasiliensis*	Quarry and ornamental pool at Rockhampton and Bajool (Qld)
Family Poeciliidae		
Green swordtail	*Xiphophorus hellerii*	Streams and rivers around Brisbane, Gladstone, between Maryborough and Cairns, Barron and Ross Rivers (northern Qld); Lake Ainsworth near Lennox Head and Burringbar Creek northern NSW; town billabong in Nhulunbuy, dam at Alice Springs and Gunn Point and waters in the vicinity of Darwin (NT); Irwin River (WA).
Platy	*Xiphophorus maculatus*	Streams, swamps and drains around Brisbane, Calliopy, Burrum Ross, Barron, Russell, Mulgrave, Tully, Johnstone and Babinda Rivers and Behana, Peewee, Louisa and Harley Creeks (northern Qld); town billabong in Nhulunbuy and Rapid Creek Darwin (NT).
Sailfin molly	*Poecilia latipinna*	Streams and rivers around Brisbane and Harvey Bay, Ross River (northern Qld), waters in the vicinity of Darwin (NT).
Guppy	*Poecilia reticulata*	Coastal drainages of Qld from Cairns to Brisbane, including the Burnett, Black

		Alice, Ross, Herbert, Fitzroy, Barron, Murray, Mossman, Mulgrave, Moresby and North and South Johnstone Rivers, Alligator and Crystal Creeks, Gustav Creek Magnetic Island, ponds and streams in Charters Towers (Qld); Billabong in Nhulunbuy, Railway Dam, Leanyer Swamp and Sadgroves Creek Darwin (NT); Roadside pool in Pilbara Drainage (WA).
Caudo	*Phalloceros caudimaculatus*	Swamps and drains around Perth, Swan-Avon Rivers; Canning River (WA).
Family Osphronemidae		
Three-spot gourami	*Trichogaster trichopterus*	Ross River and lower floodplain of the Burdekin River, Sheepstation Creek (northern Qld).
Family Cyprinidae		
Goldfish	*Carassius auratus*	Fitzroy, Dawson and Burnett Rivers in northern Qld to NSW including most coastal and inland waters of NSW, Vic. and southern Qld; Coastal drainages of south western WA between Moore, Vasse and Blackwood Rivers, Canegrass Swamp and Bromus Dam (WA); common in lowland streams (ACT); Western Plateau of SA and Coopers Creek Lake Eyre drainage (SA).
Rosy barb	*Puntius conchonius*	Streams in and south of Brisbane (Qld); Margaret River area Western Australia.
Sumatra barb	*Puntius tetrazona*	No data obtained.
Whitecloud mountain minnow	*Tanichthys albonubes*	Creek in Brisbane (Qld); Green Point Creek Central Coast, Piles Creek, Somersby (NSW).

Appendix E: Sample numbers for batch testing of imported ornamental fish

Table E1 provides the numbers of fish required to be tested from a batch to be 95 per cent confident of detecting at least one positive if the agent is present at a prevalence of 5 per cent or more if the test method has 100 per cent sensitivity and specificity. Note that for batch sizes of 19 fish or less, all fish in the batch would need to be tested.

Table E1 Sample size to detect with 95 per cent confidence the presence of an agent that is 5 per cent prevalent in a population

Population	Sample Size
19	19
20	19
21	20
22	21
23–24	22
25	23
26–27	24
28	25
29–30	26
31–32	27
33–34	28
35–36	29
37–38	30
39–40	31
41–43	32
44–45	33
46–48	34
49–51	35
52–55	36
56–58	37
59–62	38

Population	Sample Size
63–67	39
68–72	40
73–77	41
78–83	42
84–90	43
91–98	44
99–107	45
108–117	46
118–130	47
131–144	48
145–162	49
163–184	50
185–211	51
212–247	52
248–297	53
298–369	54
370–483	55
484–691	56
692–1194	57
1195–4107	58
4108–∞	59

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Glossary of abbreviations

ABARE	Australian Bureau of Agricultural and Resource Economics
ABPM	Animal Biosecurity Policy Memorandum
ACT	Australian Capital Territory
ALIV	African lampeye iridovirus
ALOP	appropriate level of protection
AQIS	Australian Quarantine and Inspection Service
ATPase	adenosine triphosphatase
ATV	Ambystoma tigrinum virus
BIV	Bohle iridovirus
BF-2	bluegill fry
CHSE-214	chinook salmon embryo
CPE	cytopathic effect
DFV	doctorfish virus
DGIV	dwarf gourami iridovirus
DNA	deoxyribonucleic acid
ECV	European catfish virus
EPC	epithelioma papilosum carpio
EHN	epizootic haematopoietic necrosis
EHNV	epizootic haematopoietic necrosis virus
ELISA	enzyme-linked immunosorbent assay
ENV	erythrocytic necrosis virus
EPBC Act 1999	*Environment Protection and Biodiversity Conservation Act 1999*
FRMA	*Fisheries Resource Management Act 1994*
ESV	European sheatfish virus
FLIV	flounder iridovirus
FV–3	frog virus 3
GFV–1	goldfish iridovirus 1

GFV–2	goldfish iridovirus 2
GIV	grouper iridovirus
GSIV	giant sea perch iridovirus
GV–6	guppy virus 6
IBC	inclusion body bearing cells
ICTV	International Committee on Taxonomy of Viruses
IFAT	indirect fluorescent antibody test
IRA	import risk analysis
ISKNV	infectious spleen and kidney necrosis virus
IRAAP	Import Risk Analysis Appeals Panel
LCDV	lymphocystis disease virus
LCDV–1	lymphocystis disease virus 1
LCDV–2	lymphocystis disease virus 2
LMBV	largemouth bass virus
MCIV	Murray cod iridovirus
MCP	major capsid protein
NACA	Network of Aquaculture Centres in Asia-Pacific
NZeelV	New Zealand eel virus
NF-H1	neurofilament triplet H1-like protein
NSW	New South Wales
OIE	World Organisation for Animal Health
OMRV	Oxyeleotris marmoratus iridovirus
PCR	polymerase chain reaction
PEV	Pelophylax esculentus virus
pfu	plaque forming units
PGIV–l	pearl gourami iridovirus 1
PIAA	Pet Industry Association of Australia Ltd
PIRSA	Department of Primary Industries and Resources of South Australia

PPIV	pike-perch iridovirus
ppm	parts per million
Qld	Queensland
RBIV	rock bream iridovirus
REV	Rana esculenta virus
RPV	Redwood Park virus
RCV–Z	Rana catesbeiana virus Z
REA	restriction enzyme analysis
RFIV	rockfish iridovirus
RFLP	restriction fragment length polymorphism
RGV	Rana grylio virus
RNA	ribonucleic acid
RRV	Regina ranavirus
RSIV	red sea bream iridovirus
RUK	Rana temporaria United Kingdom iridovirus
RTRV	Rana tigrina ranavirus
SBIV	sea bass iridovirus
SCRV	Santee-Cooper ranavirus
SERV	short-finned eel ranavirus
SGIV	Singapore grouper iridovirus
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures
TBIV	turbot iridovirus
TCID50	tissue culture infectious dose 50
TFV	tiger frog virus
WV	Wamena virus
WSIV	white sturgeon iridovirus
WTO	World Trade Organization