# FINAL REPORT

aquatic animal health subprogram

## 2009/044 Aquatic Animal Health Subprogram: surveys of ornamental fish for pathogens of quarantine significance

**Joy Becker, Anneke Rimmer, Alison Tweedie, Matt Landos, Mark Lintermans and Richard Whittington**

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**FRDC Project No. 2009/044**

# TABLE OF CONTENTS

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**PRINCIPAL INVESTIGATOR:** Dr Joy Becker
**ADDRESS:** Faculty of Veterinary Science
The University of Sydney
425 Werombi Road
Camden NSW 2570
Tel: 02 9036 7731 Fax: 02 9351 1618

**OBJECTIVES:**

1. To determine whether DGIV is entering Australia despite quarantine practices

2. To determine whether CyHV2 is entering Australia despite quarantine practices

3. To determine whether DGIV is already established in farmed gourami in Australia

4. To determine whether CyHV2 is already established in farmed goldfish in Australia

5. To determine whether DGIV is already established in wild gourami in Australia

6. To determine whether CyHV2 is already established in wild goldfish in Australia

7. To determine whether domestic goldfish free of CyHV2 succumb to disease when cohabitated with imported goldfish carrying CyHV2

8. To extend the findings of this study to the ornamental fish sector in Australia and provide information for use by DAFF

## NON TECHNICAL SUMMARY

**OUTCOMES ACHIEVED TO DATE**

This project will assist in ensuring the sustainability and profitability of the aquatic industry and the health of natural resources by providing industry and governments with knowledge of the entry of DGIV and CyHV2 in Australia. The overall outcome of this project was successful through the provision of scientific evidence of the incursions of exotic viruses from ornamental fish in Australia. This will assist in the design of improved quarantine policy for live imported ornamental fish, disease prevention strategies, improved policy regarding domestic aquaculture production and facilitate the certification of farms as being free of these viruses. Also, outcomes of this project will help protect recreational fisheries through improved conservation management of threatened freshwater fish and help promote aquaculture of native fish.

Previous R&D funded by FRDC developed molecular diagnostic tests for dwarf gourami iridovirus (DGIV) and cyprinid herpesvirus 2 (CyHV2). These viruses were considered exotic to Australia, although disease outbreaks were reported from domestic farms and the viruses were readily detected at retail outlets selling ornamental fish. This project was developed to use the validated PCR assays to determine whether DGIV and CyHV2 were in fact entering Australia despite quarantine practices and to further determine if these viruses were established in domestic populations of fish.

This project is of national significance. It was developed in close consultation with Commonwealth Department of Agriculture, Fisheries and Forestry (DAFF), the Murray Darling Basin Authority and in consultation with FRDC.

As a result of this project, DGIV was consistently found in several species of gourami imported from six different countries. The virus was also found in stocks of gourami from wholesale premises, at retail outlets and one domestic fish farm. The findings indicate that the health certification at exporting countries was insufficient to detect and prevent fish with DGIV being exported to Australia. Once fish arrive in Australia, quarantine and visual inspection were insufficient to identify fish with DGIV infections. Finally, DGIV was found in a group of platy at a domestic ornamental fish farm. At the time of collection, there was no reported outbreak and the infection was presumably sub-clinical. The detection of DGIV at a domestic farm is concerning due to the risk of spreading (and potentially amplifying) the virus through the live fish trade to other farms and retail outlets and the risk of releasing the virus into natural waterways through contaminated effluent and other waste. The lack of a plan to deal with such an incursion of an exotic pathogen is concerning. These pathways increase the opportunity for DGIV to become established in wild populations, which would impact on recreational fisheries, biodiversity and aquaculture development.

Similarly, CyHV2 was found at wholesaler premises, farms and notably in several populations of wild goldfish in the ACT and Victoria. The findings of the project demonstrated that CyHV2 was already established in Australia and were used to inform quarantine policy to revoke the requirement for goldfish exported to Australia to be certified free of CyHV2. The findings provided clear

evidence that an aquatic pathogen from ornamental fish with quarantine significance can become established in farmed and wild populations. This is of particular significance to Australia as there are many endemic and ecologically sensitive populations of fish that may be severely affected by exotic pathogens. The incursion of CyHV2 in Australia should be considered a case study to inform pathway analysis for pathogen establishment.

The findings of this project supports revision of policy to prevent incursion of exotic pathogens from imported ornamental fish and previous recommendations that laboratory testing should be carried out as an effective way of detecting exotic pathogens in imported ornamental fish. Additionally, policy revision and new policy development should address operational procedures to minimize biosecurity risks if notifiable agents are found, mandatory reporting of mortalities during quarantine, diagnostic testing of rejected consignments and increased tracking and traceability of ornamental fish. Recommendations were made to complete a risk analysis of the aquarium trade as a pathway for release of DGIV in Australia. This is important given the wide host range for DGIV and the high prevalence of the virus in retail outlets. Experimental studies are also needed to determine the range of native fish species that are susceptible to DGIV with species closely related to Murray cod given a high priority.

Recommendations for further research on the epidemiology of DGIV for freshwater fish living in Australia, sociology research to investigate the role humans play in the dispersal of pet fish in the wild and help protect future aquaculture opportunity.

In summary, this project achieved its objectives in provision of scientific data to support the revision of national policy to prevent the incursions of exotic viruses from the ornamental fish trade. This will help protect Australia's recreational fisheries through improved conservation management of freshwater fish species and help promote aquaculture.

**KEYWORDS: dwarf gourami iridovirus, cyprinid herpesvirus 2, ornamental fish, biosecurity**

**ACKNOWLEDGEMENTS**

Numerous individuals participated in this research project, and they are listed in Appendix 2, Staff.

## BACKGROUND

This project was a natural extension of FRDC 2007/007 Aquatic Animal Health Subprogram: optimisation of PCR tests for diagnosis of megalocytivirus (gourami iridovirus) and cyprinid herpesvirus 2 (goldfish herpesvirus) in which new diagnostic tests for these viruses were developed and transferred to other laboratories throughout Australia. Also, this project demonstrated that dwarf gourami iridovirus (DGIV) and cyprinid herpesvirus 2 (CyHV2) were detected in dead and moribund gourami and goldfish (*Carassius auratus*), respectively from retail outlets in NSW (Whittington et al., 2009). The current project used the validated PCR assays described in Whittington et al. (2009) in a series of epidemiological surveys to address issues related to quarantine, natural resource management and aquaculture development. The project related directly to the FRDC strategic challenge to maintain and improve the management and use of aquatic natural resources to ensure their sustainability, because:

1. native finfish species such as Murray cod (*Maccullochella peelii*) may currently be threatened by viruses suspected to be entering Australia through the trade in ornamental fish; this may prevent threatened species recovery and may also preclude successful aquaculture of these species
2. the domestic ornamental fish aquaculture industry may be threatened by the same means
3. the results of surveys for specific viruses will inform policy development, import risk analysis and directly contribute to the understanding of risk

One of the potential routes for entry into Australia for significant aquatic pathogens is via the ornamental fish trade, in which fish are imported under a policy based on a formal Import Risk Assessment (IRA). Previous studies determined that ornamental fish entering Australia may carry pathogens of quarantine concern, specifically DGIV and CyHV2. DGIV belongs to the genus *Megalocytivirus* and causes mortality outbreaks that have devastated aquaculture enterprises particularly in Asia. Also, Murray cod are highly susceptible to megalocytivirus infections following an outbreak at a farm in Victoria in 2003 that caused significant mortality (Lancaster et al., 2003). Cyprinid herpesviruses are also significant; the focus of this project was CyHV2, which infects goldfish. The first confirmation of CyHV2 in Australia was in 2003 in goldfish from a farm in Western Australia (Stephens et al., 2004). A closely related exotic virus, cyprinid herpesvirus 3 (Koi herpesvirus, KHV) causes epidemics in carp (*Cyprinus carpio*); however it has not been detected in Australia.

In September 2008, Biosecurity Australia announced (BAA 2008/29) the formal commencement of an IRA under the regulated IRA process to review Australia's freshwater ornamental finfish policy with respect to quarantine risks associated with DGIV and related viruses (see Appendix 6 for Biosecurity Australia Advice notices related to this project). The draft IRA report was open for review and comment in March 2009 (BAA 2009/06) by stakeholders. A provisional report was released in July 2010 (BAA 2010/22) and the Director of Animal and Plant Quarantine decided to await the outcomes of this project before making a policy determination (BAA 2012/01).

**NEED**

In project FRDC 2007/007 Aquatic Animal Health Subprogram: optimisation of PCR tests for diagnosis of megalocytivirus (gourami iridovirus) and cyprinid herpesvirus 2 (goldfish herpesvirus) and previous studies (Stephens et al., 2004, Go et al., 2006), it was determined that ornamental fish entering Australia may carry pathogens of quarantine concern, specifically DGIV and CyHV2. Although no pre-border survey has been conducted, the detection of exotic viruses in fish at retail outlets could be due to an infection acquired in these premises. Ornamental fish are imported under a policy based on a formal Import Risk Assessment (IRA). In September 2008, Biosecurity Australia announced the formal commencement of an IRA to review Australia's freshwater ornamental finfish policy with respect to quarantine risks associated with DGIV and related iridoviruses. Australia has imported a large number of gouramis for many decades. The 1999 IRA considered several species of gouramis and concluded that specific risk management measures were required for these species due to biosecurity risk posed by iridoviruses, including DGIV. Australia's quarantine measures include that gouramis are held in an export premises for a minimum 14 day period prior to export, health certification stating that they are sourced from populations with no known significant clinical disease in the last six months, and that the fish are held in post-arrival quarantine for a minimum of 14 days. These are the key features which need to be reviewed and additional scientific data would enhance the review. See Appendix 5 for the import conditions for live freshwater fish (excluding Salmonidae).

The developing Australian ornamental fish aquaculture industry may be at risk due to introduced pathogens. This is of particular relevance for goldfish, where domestic breeders claim that their stock succumb to diseases such as CyHV2 disease when brought into contact with imported goldfish in wholesale and retail premises. This disease agent was also specifically addressed in the 1999 IRA.

There is need to determine whether DGIV and CyHV2 are in fact entering Australia despite quarantine practices, and further, to determine whether either virus is already established in farmed or wild ornamental fish in Australia.

## OBJECTIVES

1. To determine whether DGIV is entering Australia despite quarantine practices

2. To determine whether CyHV2 is entering Australia despite quarantine practices

3. To determine whether DGIV is already established in farmed gourami in Australia

4. To determine whether CyHV2 is already established in farmed goldfish in Australia

5. To determine whether DGIV is already established in wild gourami in Australia

6. To determine whether CyHV2 is already established in wild goldfish in Australia

7. To determine whether domestic goldfish free of CyHV2 succumb to disease when cohabitated with imported goldfish carrying CyHV2

8. To extend the findings of this study to the ornamental fish sector in Australia and provide information for use by DAFF

## METHODS

The main research strategy was to conduct surveys aimed at each level in the ornamental fish industry to detect DGIV and CyHV2 by molecular techniques (PCR). We surveyed groups of ornamental fish beginning with fish that have just arrived in Australia (pre-border), those under quarantine orders, fish just released from quarantine (post border), and ornamental fish already in Australia (in retail outlets, farms and wild fish). This will provide information to determine if imported fish infected with either virus are getting past quarantine undetected and if so, are already established in the farmed and wild domestic populations.

Research assistants, Mrs. Alison Tweedie and Mrs. Rebecca Maurer were appointed to the project to undertake the molecular testing and associated laboratory work. A PhD candidate, Ms. Anneke Rimmer was recruited to the project. An aquatic animal veterinarian and project consultant, Dr Matt Landos was engaged to collect samples from retail outlets and from aquaculture premises. In consultation with AQIS, we applied for a quarantine approved premise (referred to as the USYD QAP) to allow the importation of ornamental fish to the University of Sydney (USYD).

This project has nine components, seven of which were surveys relating to Objectives 1-6. The other components were a series of experimental transmission trials in goldfish and extension and communication of the project findings to DAFF and stakeholders of the ornamental fish sector relating to Objectives 7 and 8. For clarity, the overall survey design is described below with specific detail relating to each survey presented in the appropriate section of the results.

### Survey Design and Sample Collection

With the exception of the opportunistic surveys, each survey was undertaken in specifically defined populations of fish (Table 1). Where possible, each population was tested to detect 2% prevalence with 95% confidence assuming a test of 100% sensitivity and specificity. In general this required testing at least 150 individual fish from each population. Populations were defined as groups of fish of the same species that were in close contact (e.g. sharing water, same farm location) and were collected at the same time or within the same season. For Survey 3 and 4 (Table 1), at farms with numerous ponds/tanks, a random sequence generator was used to select the aquaculture units and a small number of fish (e.g. 3 to 7) were collected via dip net from each unit to reach 150. At farms with a small number of tanks/ponds, approximately equal numbers of fish were collected from all units to reach the sample size of 150. Where possible, samples were collected four times each year to account for possible seasonal variations in prevalence of infection in order to increase the likelihood of detecting infections. It was recognised that wild fish may be difficult to procure in such numbers or in all four seasons. If only 30 fish are obtained from a population the minimum prevalence detection level becomes 10% for 95% confidence.

The data were presented as the prevalence of infection detected in each population with exact binomial confidence intervals calculated using pooled sample prevalence approaches. A one-sided 97.5% confidence interval was presented in cases where either 0% or 100% were found to be positive in a given population. If no positive samples were detected in a population the population was defined as not infected (within the assumptions specified). The presence or absence of virus in retail premises was reported. Prevalence and confidence intervals were calculated using Stata, version 10 (Stata Corporation, College Station, TX, USA). Prevalence for pooled samples was estimated under the assumptions of fixed pool size and perfect test sensitivity and specificity using Epitools

(http://epitools.ausvet.com.au/content.php?page=PooledPrevalence).

**Experimental Challenge Trials using CyHV2**

Anecdotally, it was reported that goldfish from a farm in northern NSW experienced high levels of mortality once they entered the retail environment. It was suggested that the cause of mortality was related to the pathogens associated with subclinical disease from imported goldfish. It was popularly assumed that CyHV2 was involved. CyHV2 infection has been detected in goldfish (from mixed populations of domestic and imported goldfish) purchased from retail locations (Whittington et al., 2009).

Virology techniques for CyHV2 are limited as isolates are known to quickly lose virulence and their ability to propagate in culture (Jung and Miyazaki, 1995). Therefore, bioassays were required. Methodology for *in vivo* amplification of CyHV2 PCR positive tissue was developed to attempt to obtain a virulent isolate of the virus. Goldfish from a domestic source in Survey 4 which were highly unlikely to be infected with CyHV2 were cohabitated with goldfish suspected of carrying CyHV2 in 120 L aquaria with biological filtration.

**Detection and Confirmation of DGIV**

Upon death, fish were kept frozen (mostly at -80°C) until time of processing. Fish were processed in batches to maximize efficiency of automated laboratory processes. Kidney, liver and spleen were dissected from each fish using sterile techniques. The tissue was homogenized and clarified by the Fastprep technique followed by centrifugation as described by Rimmer et al. (2012). Individually, nucleic acids were extracted from a 50 µl aliquot of the clarified tissue homogenate (1:10 w/v) using a MagMax-96 viral isolation kit (Ambion, USA) according to manufacturer's instructions. Pools of extracted nucleic acids were tested with a maximum of four individuals from the same consignment. In cases where a pool was shown to be qPCR positive, each sample was tested individually. For each sample, 1:10 clarified homogenates and excess fish tissue (where available) were placed in the -80°C archive for future retrospective testing.

For fish that were submitted fixed in ethanol, the internal organs were dissected, combined with the homogenizing medium and subjected to centrifugation. The supernatant was decanted and this step was repeated two more times to wash the ethanol from the tissues. The washed tissue was then homogenized and the DNA extracted as described above. For paraffin embedded tissues, DNA was extracted from wax shavings as described by Go et al. (2006) and purified using a High Pure Viral Nucleic acid kit (Roche). Each paraffin embedded sample was tested as a neat preparation and diluted 1:10 in molecular grade water.

The qPCR assay targeting a specific sequence of the major capsid protein (MCP) gene of megalocytiviruses was used to detect DGIV. Amplification was performed as described by Rimmer et al. (2012) using the forward and reverse primers identified as C1073 and C1074 from Whittington et al. (2009) (see Appendix 3 for details on primers). Positive samples from the USYD QAP and a domestic farm were confirmed using sequence information. For the USYD QAP samples, one to three fish with the highest viral load from each consignment were selected for confirmation. Conventional PCR (as described by Rimmer et al. 2012) was performed using primers C50 and C51 targeting a larger fragment of the MCP gene (Go et al. 2006). PCR results were assessed by electrophoresis in 2% w/v agarose gels stained with ethidium bromide. Subsequently, PCR products were purified and sent to a commercial laboratory for sequencing. Multiple sequence alignments were performed with a selection of published megalocytivirus sequences obtained from GenBank using MEGA5 (Tamura et al. 2011). For the four positive samples from the domestic farm, the products from the qPCR assay were assessed by gel electrophoresis as above, purified and sent for sequencing. Additionally, conventional PCR assays targeting the IRB6 (OIE reference assay; Kurita et al., 1998) and the ATPase genes (Go et al., 2006) were performed on the DGIV positive samples from the domestic farm and PCR products were sequenced.

**Detection and Confirmation of CyHV2**

Goldfish were kept frozen at -80°C until time of processing. Goldfish were processed in batches to maximize efficiency of automated laboratory processes. Kidney, liver and spleen were dissected from each fish using sterile techniques. The preparation of the clarified tissue homogenates and the extraction of nucleic acids were carried out as described above using the Fastprep and MagMax-96 techniques. For each sample, 1:10 clarified homogenates and excess fish tissue (e.g. gills) were placed in the -80°C archive for future retrospective testing.

A qPCR assay targeting a specific sequence of the DNA polymerase (DNApol) gene for CyHV2 was used to detect the virus. Amplification was performed as described in Whittington et al. (2009) using the forward and reverse primer identified as C1153 and C1154 (see Appendix 3 for details on primers). Typically, pools of extracted nucleic acids were tested with a maximum of five individuals from the same consignment and positive pools were re-tested

individually. However, in situations where previous testing had revealed a large proportion of positive fish (e.g. >20%), nucleic acids were tested individually in the first instance. Positive results were confirmed using a second independent PCR amplification for the helicase gene (Waltzek et al 2009).

To confirm the detection of CyHV2 at a farm in Victoria and in the wild goldfish from Cotter Reservoir, ACT PCR results were assessed by electrophoresis in 2% w/v agarose gels stained with ethidium bromide. Subsequently, PCR products were purified and sent to a commercial laboratory for sequencing. For confirmation of CyHV2 in the wild goldfish collected from Victoria, conventional PCR was performed targeting the DNApol gene using the primers C1109 (as described in Whittington et al., 2009) and C1158 (a CyHV2 specific primer). PCR results were assessed by electrophoresis in 2% w/v agarose gels stained with ethidium bromide. As above, PCR products were purified and sent to a commercial laboratory for sequencing. BLAST analyses confirmed the presence of CyHV2 sequence.

**Development of plasmid control DNA for qPCR assays**

A future development identified from FRDC 2007/007 Aquatic Animal Health Subprogram: optimisation of PCR tests for diagnosis of megalocytivirus (gourami iridovirus) and cyprinid herpesvirus 2 (goldfish herpesvirus) was to generate plasmid controls containing the key genetic elements of DGIV and CyHV2. This was necessary in the absence of cell-culture derived viral stocks to have the appropriate controls for diagnostic testing. The plasmid DNA control used with the megalocytivirus qPCR assay (*pDGIVmcp*) was prepared by cloning a sequence specific insert into plasmid pCR2.1 (Invitrogen). The sequence insert was 694 nucleotides in length and corresponded to start position 2 of the MCP gene of Murray Cod Iridovirus (AY936203). Large quantities of the plasmid were obtained by culture of transformed TOP10 *E.coli* (Invitrogen). When linearised by restriction enzyme digestion, *pDGIVmcp* is amplified efficiently by the megalocytivirus qPCR assay. The analytical sensitivity of the qPCR assay using the pDGIVmcp plasmid was 100 copies (Rimmer et al., 2012).

The plasmid DNA control used with the CyHV2 qPCR assay (*pCYHV 2DNApol*) was prepared by cloning a sequence specific insert into plasmid pCR2.1 (Invitrogen). The sequence insert was 401 nucleotides in length and corresponded to start position 29 of the DNA polymerase gene of Cyprinid Herpesvirus 2 (AY939863.1). Large quantities of the plasmid were obtained by culture of transformed TOP10 *E.coli* (Invitrogen). When linearised by restriction enzyme digestion, *pCYHV 2DNApol* is amplified efficiently by the CyHV2 qPCR assay. The analytical sensitivity of the qPCR assay using the plasmid was 100 copies.

Each plasmid control provides an appropriate external standard for comparison of qPCR assay results obtained at different times and on different PCR machines. Standard curves prepared by amplification of a dilution series of known quantities of plasmid DNA are suitable for quantification of DGIV and CyHV2 in fish tissue samples.

Table 1. Summary of epidemiology surveys to determine if DGIV and CyHV2 are present in the Australian ornamental fish industry.

| Survey ID | Survey aim (Project Objective No.) | Study population | Sample collection | Target no. of fish per sample/population |
|---|---|---|---|---|
| 1a | To determine if DGIV is present in gourami species upon initial arrival at a QAP premise (1) | imported species of gourami | consignments of gourami were imported directly to the USYD QAP and fish were sampled on the day of importation (pre-border) | 150 individuals per species per sample period |
| 1b | To determine if CyHV2 is present in goldfish upon initial arrival at a QAP premise (2) | imported goldfish | not done as CyHV2 was detected in farmed and wild goldfish prior to the commencement of this survey and the virus is now considered endemic | |
| 1c | To provide documentation of the detection of DGIV from moribund fish in quarantine (1) | consignments of ornamental fish with suspect megalocytivirus infections submitted by AQIS to Fisheries WA laboratories | tissue embedded in wax blocks or from ethanol-fixed whole fish was submitted by Fisheries WA (pre-border) | opportunistic sampling |
| 2a | To determine if DGIV is present in gourami species following the quarantine period (1) | imported species of gourami that have recently completed the quarantine period | consignments of gourami were sent to USYD within one week of completing quarantine and sampled on receival (post-border) | 150 individuals per species per sample period |
| 2b | To determine if CyHV2 is present in goldfish following the quarantine period (2) | imported goldfish that recently completed the quarantine period | not done as CyHV2 was detected in farmed and wild goldfish prior to the commencement of this survey and the virus is now considered endemic | |
| 3 | To determine if DGIV is established in farmed gourami (3) | domestically produced gourami or other species of ornamental fish with a prior history of DGIV or iridovirus infections | small numbers of individual fish were collected from randomly selected aquaculture units (ponds/tanks) at each enterprise (domestic farmed) | 150 individuals per species per sample period |
| 4 | To determine if CyHV2 is established in farmed goldfish (4) | domestically produced goldfish | small numbers of individual fish were collected from randomly selected aquaculture units (ponds/tanks) at each enterprise (domestic farmed) | 150 individuals per sample period |
| 5 | To determine if DGIV is established in wild gourami (5) | wild (feral) gourami species | electrofishing techniques were used in areas of known feral populations of gourami (domestic wild) | 150 individuals per species per population |
| 6 | To determine if CyHV2 is established in wild goldfish (6) | wild (feral) goldfish | Electrofishing and netting techniques were used in areas of known feral populations goldfish and frozen goldfish were submitted by fisheries biologists from Victoria (domestic wild) | 150 individuals per population |
| 7 | To provide further documentation of the detection of DGIV and CyHV2 from retail outlets (2) | dead and moribund ornamental fish from retail outlets | retail staff placed dead/moribund fish in a domestic freezer | opportunistic sampling |

## RESULTS AND DISCUSSION

For clarity, the results of the seven surveys have been divided into sections dealing with each virus.

*I. Results relating to the detection of DGIV*

Objectives 1, 3 and 5 were developed to determine if DGIV was indeed going undetected through quarantine and if so, to assess to what extent the virus has spread through the Australian ornamental fish industry. Surveys 1a, 1c, 2a, 3, 5 and 7 relate to these objectives (Table 1).

The project met with considerable opposition from the ornamental fish industry, including the Aquatic Policy Group of The Pet Association of Australia. As such, we were unable to find an existing wholesaler with a quarantine approved premise (QAP) that was willing to import fish for the project. It was decided in consultation with AQIS that the Infectious Disease Laboratory, headed by Professor Whittington would apply to become a QAP (referred to as the USYD QAP). This allowed us to receive fish that were under quarantine orders with our own import permit. The application and assessment process for a QAP begin in February 2010 and was approved in October 2010.

For Survey 1a, a total of 32 ornamental fish exporters from seven different countries were contacted via email requesting to purchase gourami of various species that could be shipped to Australia. We received a response from 14 suppliers and then subsequently only four of these suppliers continued to engage in email communication that culminated in the purchase of fish. A total of six consignments of ornamental fish were imported directly to the USYD QAP. Upon receival, fish were euthanized and held at -80°C. Six separate consignments containing a variety of gourami species were received between February 2011 and November 2011. One consignment was received from Indonesia, Sri Lanka and Thailand and three consignments from the same exporter from Singapore (Table 2).

A total of 2086 gourami were imported to the USYD QAP and tested for the detection of DGIV by qPCR. Positive fish were found in all six consignments. Nearly 19% of the total fish population were positive for DGIV (Table 2, Figure 1). For all consignments, the highest prevalence of DGIV was detected in Blue/gold gourami (*Trichogaster trichopterus*) at 29%, followed by Kissing gourami (*Helostoma temminkii*) at 19% (Table 3). The lowest level of DGIV detection was found in Thick lipped gourami (*Colisa labiosa*); however this species was obtained from only one exporting country. The Indonesian consignment contained the highest prevalence with over 50% of the fish being positive for DGIV (Table 2). The Sri Lankan consignment had the lowest prevalence with one of 308 fish being positive. The other consignments from Singapore and Thailand had intermediate detection levels for DGIV between 6% and 20% (Table 2).

A 399 bp fragment of the MCP gene was successfully amplified in DNA extracts from one fish from four of six consignments received at the USYD QAP (Figure 2). The samples were from a Blue/gold and Kissing gourami from Singapore, a Pearl gourami from Indonesia and a Kissing gourami from Thailand. Nucleotide sequences were obtained and compared with other megalocytiviruses with published sequences in GenBank. All four isolates had nucleotide sequence identities of 100% with each other and all other ISKNV-like megalocytivirus sequences included in the analysis (Figure 3). This confirmed the detection of DGIV. The conventional PCR is significantly less sensitive than the qPCR (Rimmer et al. 2012) and this is the most likely reason for the lack of amplification in the other fish from the Singapore and the Sri Lanka consignments.

For Survey 1c, a total of 51 individuals which represented six separate species were collected during the quarantine period and submitted from WA Fisheries (Table 4). DGIV DNA was successfully extracted and amplified from both paraffin and ethanol-fixed fish tissues. DGIV was confirmed in a large proportion of Red tiger oscar (*Astronotus ocellatus*), Dwarf gourami (*Colisa lalia*) and Blue ram (*Microgeophagus ramirezi*) (Table 4).

From Survey 2a, six consignments of various gourami species from four different wholesale enterprises were obtained for DGIV testing (Table 5). DGIV was readily detected in five of six consignments. The total prevalence for the positive consignments ranged from a low of 5.7% (1.6-14.0; 95% CI) to a high of 36.2% (28.6-44.4; 95% CI). The consignment where DGIV was not detected consisted of both Dwarf and Honey gourami (*Colisa chuna*) with the upper limit equal to 4.6% (Table 5). For all consignments from the survey of wholesalers, the highest prevalence of DGIV was detected in Pearl gourami (*Trichogaster leeri*) at nearly 30%, followed by Blue/gold gourami at 19% (Table 3). At least one individual from each species of gourami tested in this survey was positive for DGIV with the exception of the two consignments of Honey gourami (Table 3).

From the survey of domestic ornamental fish producers (Survey 3), a total of three farms were tested for evidence of DGIV. The two farms based in NSW produced goldfish and guppies (*Poecilia reticulata*), while the farm from Queensland produced goldfish, a variety of gourami species and platy (*Xiphophorus maculatus*) (Table 6). DGIV was detected in 4 of the 140 platy sampled from the farm in Queensland (Figure 4, 5; Table 6). The detection of DGIV was confirmed with all four isolates from the farmed platy having identical sequences within the primer regions for the MCP gene fragment (167 bp) with each other and other ISKNV-like megalocytiviruses available in GenBank (Figure 6). Only two of four platy isolates could be amplified using conventional PCR targeting the IRB6 and ATPase genes. Sequencing of these PCR products confirmed the previous results as being an ISKNV-like megalocytivirus. As DGIV is nationally notifiable, a detailed laboratory report was provided to the submitting veterinarian, the CVO offices for Queensland and NSW and DAFF. Sample material from each positive platy was submitted to the Australian Fish Disease Laboratory at AAHL for testing and confirmed our results. The gourami species from the Queensland farm were held at a

separate location and all tested negative but samples sizes were low. Three consignments of guppy were collected from two different farms in NSW (Table 6). All 89 guppies that were tested were considered negative for DGIV.

For the survey of wild populations of fish (Survey 5), Blue/gold gourami were collected in Queensland from locations located around Sheepstation Creek, approximately 65 km east-southeast of Townsville and the nearby lower Burdekin delta. In 2011, two seasonal samplings were completed, with a total of 668 fish available for testing. All fish were negative for the detection of DGIV (Table 7).

Small numbers of ornamental fish of nine different species were collected for the opportunistic survey of retail outlets (Survey 7). DGIV was detected in six species, including Dwarf gourami, Sailfin molly (*Poecilia latipinna*), Guppy, Molly (*Poecilia sphenops*), Angelfish (*Pterophyllum scalare*) and Platy (Table 8). These results are in alignment with the findings from Whittington et al. (2009).

Table 2. Summary of ornamental fish that were imported directly to the USYD QAP for the molecular detection of DGIV (Survey 1a).

| Export country (USYD accession no.) | Date received at USYD | Species | Common name | Mean weight ± SE (g) | Mean total length ± SE (mm) | Proportion of qPCR positive fish | Percent positive (95% confidence interval) | Proportion of consignment positive (%; 95% CI) |
|---|---|---|---|---|---|---|---|---|
| Indonesia (11/071) | March 2011 | *Colisa lalia* | Dwarf gourami | 1.3 ± 0.0 | 38.0 ± 0.3 | 104/154 | 67.5 (4.9-59.5) | 191/368 |
| | | *Trichogaster leeri* | Pearl gourami | 2.3 ± 0.0 | 54.0 ± 0.5 | 37/108 | 34.3 (25.4-44.0) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 3.5 ± 0.2 | 58.1 ± 1.4 | 50/106 | 47.2 (7.1-37.4) | (51.9; 46.7-57.1) |
| Singapore 1 (11/048) | February 2011 | *Colisa lalia* | Dwarf gourami | 3.0 ± 0.0 | 49.6 ± 0.2 | 0/97 | 0 (0-3.7) | 19/312 |
| | | *Helostoma temminkii* | Kissing gourami | 1.0 ± 0.0 | 50.0 ± 0.0 | 0/51 | 0 (0-7.0) | |
| | | *Trichogaster leeri* | Pearl gourami | 1.9 ± 0.0 | 53.4 ± 0.4 | 0/55 | 0 (0-6.5) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 3.1 ± 0.1 | 57.4 ± 0.6 | 19/109 | 17.4 (5.9-10.8) | (6.1; 3.7-9.3) |
| Singapore 2 (11/062) | March 2011 | *Colisa lalia* | Dwarf gourami | 2.7 ± 0.0 | 47.4 ± 0.2 | 1/102 | 1.0 (0-5.3) | 52/308 |
| | | *Helostoma temminkii* | Kissing gourami | 1.5 ± 0.0 | 45.2 ± 0.3 | 3/51 | 5.9 (1.2-16.2) | |
| | | *Trichogaster leeri* | Pearl gourami | 1.5 ± 0.0 | 49.2 ± 0.5 | 0/53 | 0 (0-6.7) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 4.1 ± 0.1 | 66.4 ± 0.5 | 48/102 | 47.1 (37.1-7.2) | (16.9; 6.2-12.9) |
| Singapore 3 (11/232) | November 2011 | *Colisa lalia* | Dwarf gourami | 2.6 ± 0.0 | 48.2 ± 0.2 | 1/102 | 1.0 (0.02-5.3) | 59/302 |
| | | *Helostoma temminkii* | Kissing gourami | 1.4 ± 0.0 | 46.5 ± 0.4 | 36/51 | 70.6 (2.5-56.2) | |
| | | *Trichogaster leeri* | Pearl gourami | 2.2 ± 0.0 | 57.8 ± 0.3 | 11/49 | 22.4 (6.6-11.8) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 6.2 ± 0.1 | 74.1 ± 0.4 | 11/100 | 11.0 (5.6-18.8) | (19.5; 4.5-15.2) |
| Sri Lanka (11/070) | March 2011 | *Colisa lalia* | Dwarf gourami | 1.8 ± 0.0 | 41.7 ± 0.4 | 0/100 | 0 (0-3.6) | 1/308 |
| | | *Helostoma temminkii* | Kissing gourami | 5.7 ± 0.1 | 68.4 ± 0.5 | 1/50 | 2.0 (0.05-10.7) | |
| | | *Trichogaster leeri* | Pearl gourami | 2.2 ± 0.0 | 53.7 ± 0.6 | 0/56 | 0 (0-6.4) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 2.8 ± 0.1 | 57.2 ± 0.5 | 0/102 | 0 (0-3.6) | (0.3; 0.01-1.8) |
| | | *Colisa labiosa* | Thick lipped gourami | 0.4 ± 0.0 | 28.4 ± 0.2 | 9/134 | 6.7 (3.1-12.4) | |
| Thailand (11/216) | October 2011 | *Colisa lalia* | Dwarf gourami | 0.7 ± 0.0 | 31.7 ± 0.1 | 4/144 | 3.5 (1.1-7.9) | 70/488 |
| | | *Helostoma temminkii* | Kissing gourami | 2.4 ± 0.1 | 53.7 ± 0.5 | 8/53 | 15.1 (6.7-27.6) | |
| | | *Trichogaster leeri* | Pearl gourami | 1.3 ± 0.0 | 49.0 ± 0.4 | 0/72 | 0 (0-5.0) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 5.7 ± 0.3 | 75.4± 1.1 | 48/85 | 56.5 (7.2-45.3) | (14.3; 7.8-11.4) |

Table 3. For each species, the total proportion of DGIV positive fish for all consignments collected prior to and immediately following quarantine (Survey 1a and 2a).

| Survey location and ID | Species | Common name | No. of consignments | Proportion of qPCR positive fish | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|
| USYD QAP 1a | *Colisa labiosa* | Thick lipped gourami | 1 | 9/134 | 6.7 (3.1-12.4) |
| | *Colisa lalia* | Dwarf gourami | 6 | 110/699 | 15.7 (13.1-18.7) |
| | *Helostoma temminkii* | Kissing gourami | 5 | 48/256 | 18.8 (14.2-24.1) |
| | *Trichogaster leeri* | Pearl gourami | 6 | 48/393 | 12.2 (9.1-15.9) |
| | *Trichogaster trichopterus* | Blue/gold gourami | 6 | 176/604 | 29.1 (25.5-32.9) |
| Wholesaler 2a | *Colisa chuna* | Honey gourami | 2 | 0/59 | 0 (0-6.1) |
| | *Colisa labiosa* | Thick lipped gourami | 1 | 6/51 | 11.8 (4.4-23.9) |
| | *Colisa lalia* | Dwarf gourami | 6 | 7/217 | 3.2 (1.3-6.5) |
| | *Helostoma temminkii* | Kissing gourami | 2 | 3/30 | 10.0 (2.1-26.5) |
| | *Trichogaster leeri* | Pearl gourami | 4 | 48/161 | 29.8 (22.9-37.5) |
| | *Trichogaster microlepis* | Moonlight gourami | 1 | 3/20 | 15.0 (3.2-38.0) |
| | *Trichogaster trichopterus* | Blue/gold gourami | 3 | 44/231 | 19.0 (14.2-24.7) |

Table 4. Summary of the ornamental fish that were submitted as tissues fixed in paraffin or ethanol from Fisheries WA for the detection of DGIV. The samples were originally from consignments experiencing clinical signs and mortality that were submitted by AQIS for testing during the quarantine period (Survey 1c).

| Species | Common name | USYD accession no. | Date received at USYD | Tissue type | Proportion of qPCR positive fish | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|---|
| *Astronotus ocellatus* | Red tiger oscar | 10/219 | 16/11/10 | ethanol | 13/19 | 68.4 (43.5–87.4) |
| *Colisa lalia* | Dwarf gourami | 10/134 | 06/07/2010 | paraffin | 2/3 | 66.7 (9.4-99.2) |
| *Laetacara curviceps* | Dwarf flag cichlid | 10/046 | 23/02/2010 | paraffin | 0/1 | 0 (0-97.5) |
| *Metynnis argenteus* | Silver dollar | 10/046 | 23/02/2010 | paraffin | 0/1 | 0 (0-97.5) |
| *Microgeophagus ramirezi* | Blue ram | 10/219 | 16/11/10 | ethanol | 20/26 | 76.9 (56.3-91.0) |
| Genus *Pelvicachromis* | Kribensis | 10/046 | 23/02/2010 | paraffin | 0/1 | 0 (0-97.5) |

Table 5. Summary of the ornamental fish that were purchased from wholesalers following quarantine for the molecular detection of DGIV (Survey 2a).

| Wholesaler ID[1] (USYD Accession) | Date received at USYD | Species | Common name | Mean weight ± SE (g) | Mean total length ± SE (mm) | Proportion of qPCR positive fish | Percent positive (95% confidence interval) | Proportion of consignment positive (%; 95% CI) |
|---|---|---|---|---|---|---|---|---|
| 1 (10/131) | July 2010 | *Colisa lalia* | Dwarf gourami | 2.5 ± 0.1 | 50.1 ± 0.4 | 2/50 | 4 (0.5-13.7) | 55/152 (36.2; 28.6-44.4) |
| | | *Trichogaster leeri* | Pearl gourami | 1.7 ±. 01 | 55.5 ± 0.7 | 35/51 | 68.6 (54.1-80.9) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 1.5 ± 0 | 49.4 ± 0.7 | 18/51 | 35.5 (22.4-49.9) | |
| 1 (11/239) | November 2011 | *Colisa labiosa* | Thick lipped gourami | 0.9 ± 0.0 | 37.0 ± 0.2 | 6/51 | 11.8 (4.4-23.9) | 37/271 (13.7; 9.8-18.3) |
| | | *Colisa lalia* | Dwarf gourami | 2.7 ± 0.1 | 51.4 ± 0.6 | 4/50 | 8.0 (2.2-19.2) | |
| | | *Helostoma temminkii* | Kissing gourami | 1.7 ± 0.1 | 47.0 ± 0.9 | 3/20 | 15 (3.2-37.9) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 2.7 ± 0.1 | 56.9 ± 0.4 | 24/150 | 16 (10.5-22.9) | |
| 2 (Singapore; 10/139) | July 2010 | *Colisa lalia* | Dwarf gourami | 2.8 ± 0.1 | 51.6 ± 0.6 | 1/17 | 5.9 (0.2-28.7) | 10/113 (8.8; 4.3-15.7) |
| | | *Trichogaster leeri* | Pearl gourami | 3.2 ± 0.1 | 62.5 ± 0.7 | 7/66 | 10.6 (4.4-20.6) | |
| | | *Trichogaster trichopterus* | Blue/gold gourami | 2.9 ± 0.1 | 59.6 ± 0.7 | 2/30 | 6.7 (0.1-22.1) | |
| 2 (10/172) | September 2010 | *Colisa chuna* | Honey gourami | 1.7 ± 0.1 | 41.8 ± 0.7 | 0/20 | 0 (0-16.8) | 5/83 (6.0: 2.0-13.5) |
| | | *Colisa lalia* | Dwarf gourami | 2.9 ± 0.1 | 52.3 ± 0.6 | 0/20 | 0 (0-52.2) | |
| | | *Helostoma temminkii* | Kissing gourami | 1.9 ± 0.0 | 47.7 ± 0.8 | 0/10 | 0 (0-30.9) | |
| | | *Trichogaster leeri* | Pearl gourami | 3.8 ± 0.2 | 67.0 ± 1.3 | 2/14 | 14.3 (1.8-42.8) | |
| | | *Trichogaster microlepis* | Moonlit gourami | 3.6 ± 0.2 | 68.7 ± 1.3 | 3/20 | 15.0 (3.2-38.0) | |
| 3 (Malaysia; 10/141) | July 2010 | *Colisa lalia* | Dwarf gourami | 1.8 ± 0.1 | 42.4 ± 0.6 | 0/40 | 0 (0-8.8) | 4/70 (5.7; 1.6-14.0) |
| | | *Trichogaster leeri* | Pearl gourami | 8.0 ± 6.7 | 47.0 ± 1.2 | 4/30 | 13.3 (3.8-30.7) | |
| 4 (11/231) | November 2011 | *Colisa chuna* | Honey gourami | 0.7 ± 0.0 | 30.5 ± 0.3 | 0/39 | 0 (0-9.0) | 0/79 (0; 0-4.6) |
| | | *Colisa lalia* | Dwarf gourami | 3.2 ± 0.1 | 52.2 ± 0.4 | 0/40 | 0 (0-8.8) | |

[1] export country was provided by the wholesaler and could not be verified

Table 6. Summary of ornamental fish collected from domestic aquaculture producers that were submitted for DGIV testing by qPCR (Survey 3).

| Farm ID and location[1] | USYD Lab accession | Date collected | Species | Common name | Mean weight ± SE (g) | Mean total length ± SE (mm) | Proportion of qPCR positive fish[1] | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|---|---|---|
| 2 Northern NSW | 10/109 | May 2010 | *Poecilia reticulata* | Guppy | 1.3 ± 0.1 | 45.8 ± 1.4 | 0/30 | 0 (0-11.6) |
| | 10/165 | August 2010 | *Poecilia reticulata* | Guppy | 0.8 ± 0.2 | 36.1 ± 2.8 | 0/20 | 0 (0-21.8) |
| 3 Central Coast NSW | 10/213 | October 2010 | *Poecilia reticulata* | Guppy | 0.3 ± 0.0 | 30.0 ± 0.0 | 0/44 | 0 (0-8.0) |
| 4 South East QLD | 11/190 | June 2011 | *Colisa chuna* | Honey gourami | 0.5 ± 0.1 | 25.3 ± 1.4 | 0/15 | 0 (0-21.8) |
| | | | *Colisa lalia* | Dwarf gourami | 0.8 ± 0.1 | 30.4 ± 0.7 | 0/44 | 0 (0-8.0) |
| | | | *Helostoma temminkii* | Kissing gourami | 0.9 ± 0.1 | 36.9 ± 1.1 | 0/15 | 0 (0-21.8) |
| | | | *Trichogaster leeri* | Pearl gourami | 1.3 ± 0.1 | 43.1 ± 1.8 | 0/15 | 0 (0-21.8) |
| | | | *Trichogaster trichopterus* | Blue/gold gourami | 1.0 ± 0.1 | 35.4 ± 1.2 | 0/47 | 0 (0-7.5) |
| | | | *Xiphophorus maculatus* | Platy | 1.5 ± 0.1 | 39.1 ± 0.4 | 4/140 | 2.9 (0.78-7.2) |

[1] farm ID numbers correspond and continue from Table 10

Table 7. Summary of ornamental fish collected from wild populations that were submitted for DGIV testing by qPCR (Survey 5).

| Location | USYD lab accession | Date collected | Species | Common name | Mean weight ± SE (g) | Mean total length ± SE (mm) | Proportion of qPCR positive fish | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|---|---|---|
| Queensland | 11/075 | March 2011 | *Trichogaster trichopterus* | Blue/gold gourami | 7.6 ± 0.1 | 73.8 ± 1.5 | 0/479 | 0 (0-0.77) |
| Queensland | 11/167 | August 2011 | *Trichogaster trichopterus* | Blue/gold gourami | 5.9 ± 0.1 | 68.7 ± 0.6 | 0/189 | 0 (0-1.93) |

Table 8. Summary of ornamental fish collected from retail outlets for DGIV testing by qPCR (Survey 7).

| Species | Common name | USYD lab accession | Date collected | Location of retailer | Origin of fish[1] | Proportion of PCR positive fish | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|---|---|
| *Balantiocheilus melanopterus* | Silver shark | 10/156 | 30 July 2010 | Northern NSW | Melbourne | 0/7 | 0 |
| *Colisa chuna* | Honey gourami | Various | 2008 | Various | Unknown | 0/4 | 0 (0-60.2) |
| *Colisa lalia* | Dwarf gourami | 10/158 | 30 July 2010 | Northern NSW | Melbourne | 2/2 | 100 (15.8-100) |
| *Colisa lalia* | Dwarf gourami | 10/065 | 18 March 2010 | Sydney | Sydney | 7/10 | 70 (34.8-93.3) |
| *Colisa lalia* | Dwarf gourami | 11/006 | 30 November 2010 | Northern NSW | Melbourne | 1/2 | 50 (1.2-98.7) |
| *Colisa lalia* | Dwarf gourami | Various | 2008 | Various | Unknown | 5/11 | 45.5 (16.7-76.6) |
| *Colisa lalia* | Dwarf gourami | 10/159 | 16 July 2010 | Northern NSW | Melbourne and Brisbane | 0/1 | 0 (0-97.5) |
| *Colisa lalia* | Dwarf gourami | 10/156 | 30 July 2010 | Northern NSW | Melbourne | 0/1 | 0 (0-97.5) |
| *Colisa lalia* | Dwarf gourami | 10/157 | 13 August 2010 | Northern NSW | Melbourne | 0/2 | 0 (0-84.1) |
| *Poecilia latipinna* | Sailfin molly | 10/157 | 13 August 2010 | Northern NSW | Melbourne | 1/15 | 6.7 (0.16-31.9) |
| *Poecilia latipinna* | Sailfin molly | 10/156 | 30 July 2010 | Northern NSW | Melbourne | 0/5 | 0 (0-52.2) |
| *Poecilia reticulata* | Guppy | 11/191 | 8 February 2010 | QLD | Queensland | 2/5 | 40 (5.3-85.3) |
| *Poecilia reticulata* | Guppy | Various | 2008 | Various | Unknown | 1/23 | 4.3 (0.11-21.9) |
| *Poecilia reticulata* | Guppy | 10/159 | 16 July 2010 | Northern NSW | Melbourne and Brisbane | 0/2 | 0 (0-84.1) |
| *Poecilia reticulata* | Guppy | 11/008 | 4 November 2010 | Northern NSW | Domestic | 0/1 | 0 (0-97.5) |
| *Poecilia reticulata* | Guppy | 11/010 | 4 November 2010 | Northern NSW | Domestic | 0/2 | 0 (0-84.1) |
| *Poecilia reticulata* | Guppy | 11/011 | 11 December 2010 | Northern NSW | Domestic | 0/10 | 0 (0-30.8) |
| *Poecilia sphenops* | Molly | 10/158 | 30 July 2010 | Northern NSW | Melbourne | 2/2 | 100 (15.8-100) |
| *Poecilia sphenops* | Molly | 10/156 | 30 July 2010 | Northern NSW | Melbourne | 1/4 | 25 (0.63-80.6) |
| *Poecilia sphenops* | Molly | 10/157 | 13 August 2010 | Northern NSW | Melbourne | 1/5 | 20 (0.51-71.6) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Poecilia sphenops* | Molly | 10/159 | 16 July 2010 | Northern NSW | Melbourne and Brisbane | 0/1 | 0 (0-97.5) |
| *Pterophyllum scalare* | Angelfish | 10/163 | 5 July 2010 | Northern NSW | Unknown | 1/1 | 100 (2.5-100) |
| *Pterophyllum scalare* | Angelfish | 10/157 | 13 August 2010 | Northern NSW | Melbourne | 1/3 | 33.3 (0.84-90.6) |
| *Pterophyllum scalare* | Angelfish | 10/156 | 30 July 2010 | Northern NSW | Melbourne | 0/6 | 0 (0-45.9) |
| *Trichogaster trichopterus* | Blue/gold gourami | Various | 2008 | Various | Unknown | 0/3 | 0 (0-70.8) |
| *Trichogaster trichopterus* | Blue/gold gourami | 10/159 | 16 July 2010 | Northern NSW | Melbourne and Brisbane | 0/1 | 0 (0-97.5) |
| *Trichogaster trichopterus* | Blue/gold gourami | 11/006 | 30 November 2010 | Northern NSW | Melbourne | 0/1 | 0 (0-97.5) |
| *Xiphophorus maculatus* | Platy | 10/158 | 30 July 2010 | Northern NSW | Melbourne | 6/6 | 100 (54.1-100) |
| *Xiphophorus maculatus* | Platy | Various | 2008 | Various | Unknown | 4/7 | 57 (18.4-90.1) |
| *Xiphophorus maculatus* | Platy | 10/157 | 13 August 2010 | Northern NSW | Melbourne | 0/7 | 0 (0-41.0) |
| *Xiphophorus maculatus* | Platy | 10/162 | 13 August 2010 | Northern NSW | Unknown | 0/2 | 0 (0-84.1) |

[1] origin is based on information provided by the retail outlet and cannot be verified

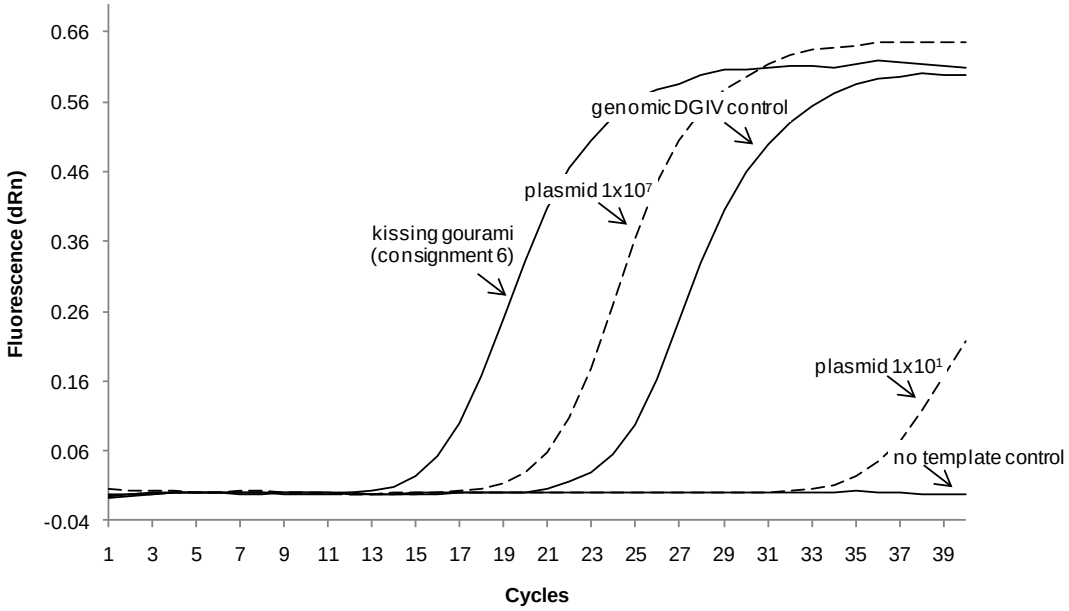


Figure 1. Amplification curves (average for duplicate reactions) for *pDGIV-MCP1* plasmid DNA (showing copies of starting template), genomic DGIV control DNA and a DGIV positive Kissing gourami (*Helostoma temminkii*) imported with Consignment 6.

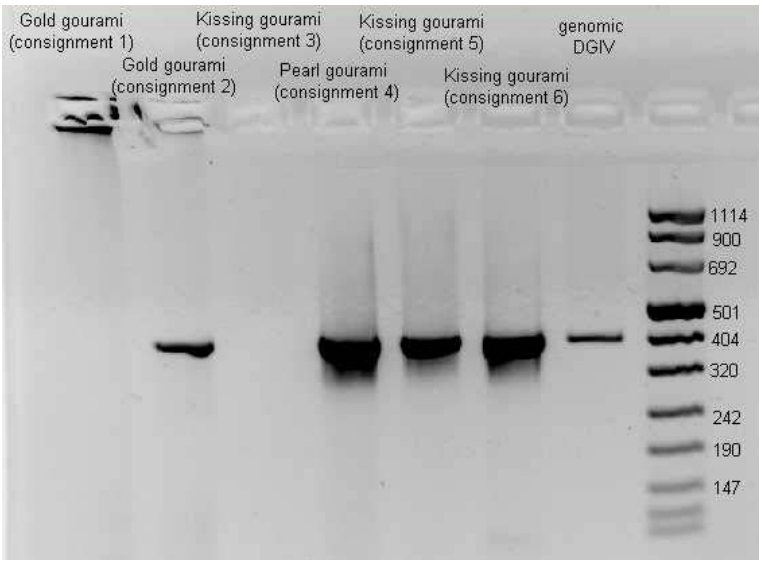


Figure 2. Agarose gel electrophoresis of DGIV DNA in tissues of imported gourami. Amplicons of 399 bp are present in gourami from Consignments 2, 4, 5 and 6.

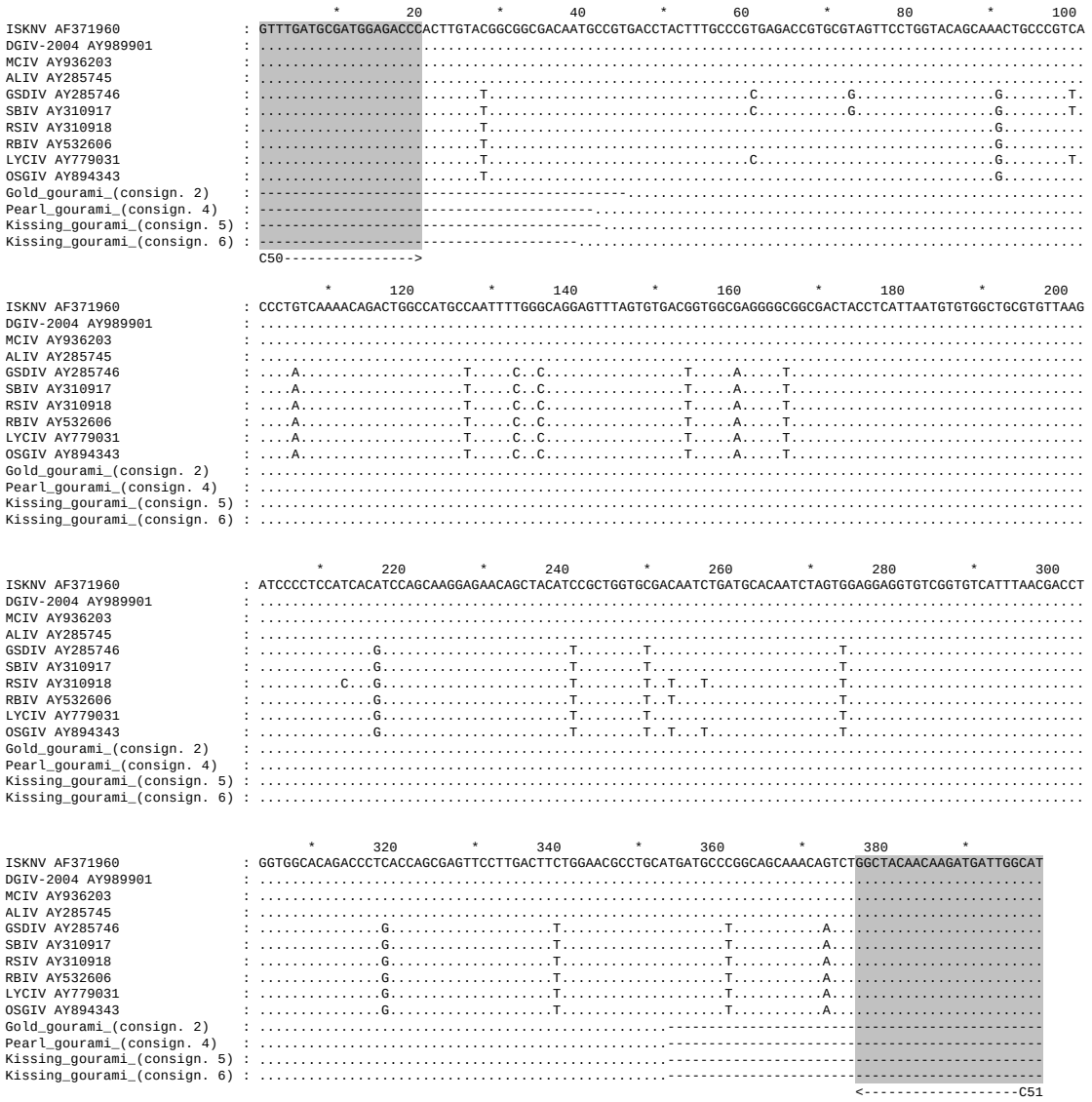


Figure 3. Nucleotide sequence of the partial major capsid protein (MCP) gene for PCR positive imported gourami, using the primers shaded in grey, with published megalocytivirus sequence *Infectious spleen and kidney necrosis virus* (ISKNV) and other published megalocytivirus sequences. Sequence acronyms are presented in Figure 6.

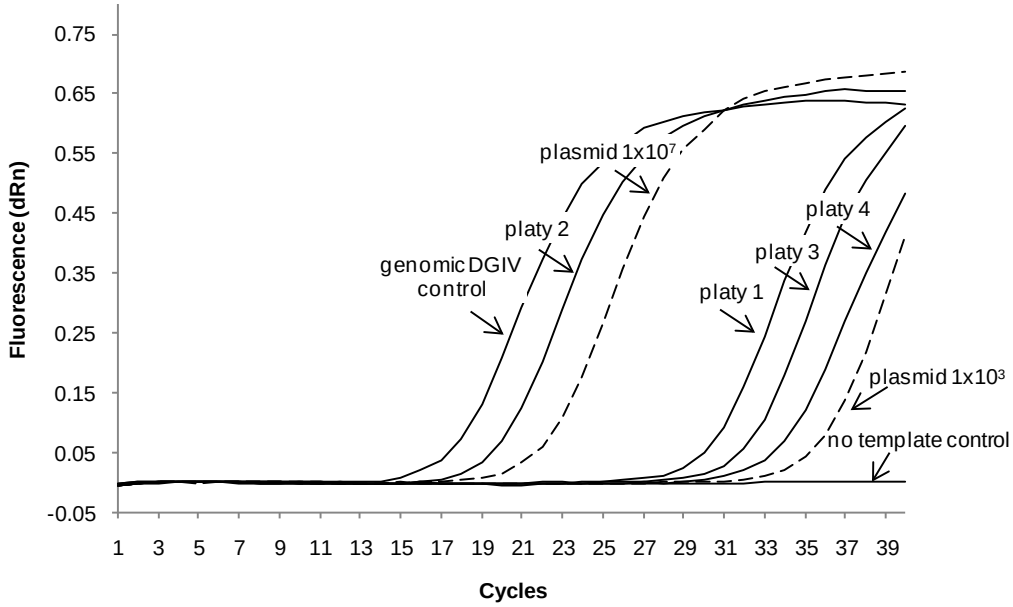


Figure 4. Amplification curves (average for duplicate reactions) for *pDGIV-MCP1* plasmid DNA (showing copies of starting template), genomic DGIV control DNA and a DGIV positive samples (SVC 11/190: 20.6, 20.19, 20.23 and 20.24) from the farmed Platy (*Xiphophorus maculatus*).

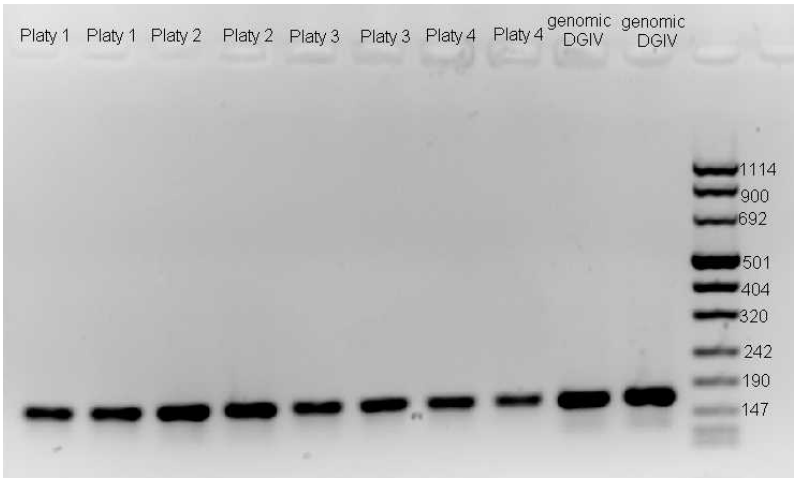


Figure 5. Agarose gel electrophoresis of DGIV DNA in tissues of farmed Platy (*Xiphophorus maculatus*). (SVC 11/190: 20.6, 20.9, 20.23 and 20.24) amplified by qPCR (as above). Amplicons of 167 bp are present in all DGIV positive samples and the corresponding genomic DGIV control DNA.

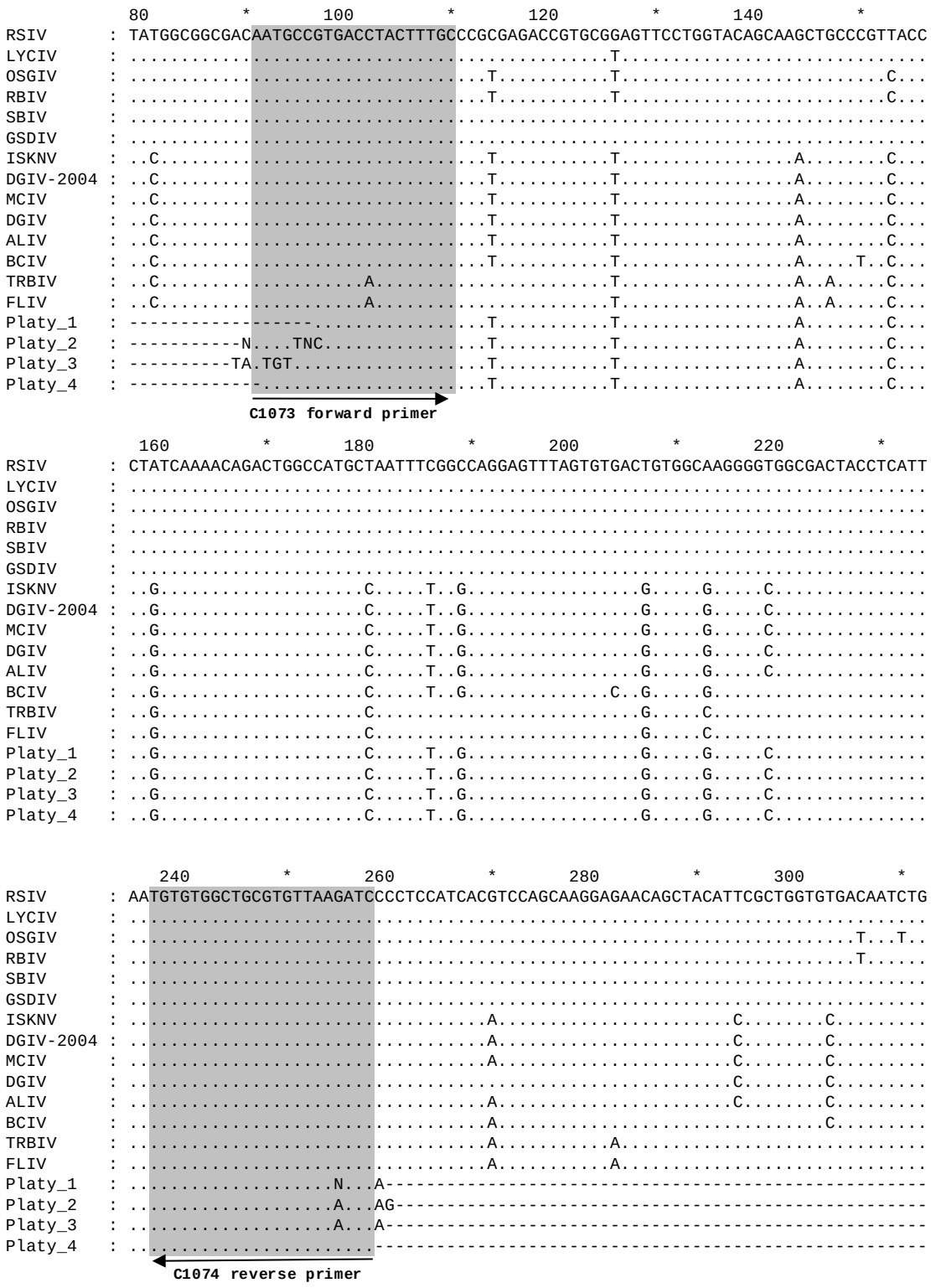


Figure 6. Nucleotide sequence alignments for the MCP gene fragment of the qPCR positive platy (*Xiphophorus maculatus*) with published megalocytivirus reference isolates.

RSIV = red sea bream iridovirus (GenBank accession: AB104413)
LYCIV = large yellow croaker iridovirus (GenBank accession: AY779031)
OSGIV = orange spotted grouper iridovirus (GenBank accession: AY894343)

RBIV = rock bream iridovirus (GenBank accession: AY532606)
SBIV = sea bass iridovirus (GenBank accession: AY310917)
GSDIV = grouper sleepy disease iridovirus (GenBank accession: AY285746)
ISKNV = infectious spleen and kidney necrosis virus (GenBank accession: AF371960)
DGIV–2004 = dwarf gourami iridovirus (GenBank accession: AY989901)
MCIV = Murray cod iridovirus (GenBank accession: AY936203)
DGIV = dwarf gourami iridovirus (GenBank accession: AY295744)
ALIV = African lampeye iridovirus (GenBank accession: AY285745)
BCIV = Banggai cardinalfish iridovirus (GenBank accession: EU753255)
TRBIV = turbot reddish body iridovirus (GenBank accession: GQ273492)
FLIV = flounder iridovirus (GenBank accession: AY633992)

*II. Results relating to the detection of CyHV2*

Objectives 2, 4 and 6 were developed to determine if CyHV2 was passing undetected through quarantine and then to assess to what extent the virus had been spread throughout goldfish populations. Surveys 1b, 2b, 4, 6 and 7 relate to these objectives (Table 1).

The initiation of Survey 1b was delayed due the considerable industry opposition and being unable to find a wholesaler with an existing QAP that would import fish to support the project. As described above, the decision to import ornamental fish directly from overseas was made quickly in consultation with DAFF and AQIS. The application process took nearly one year and was granted in October 2010.

During this time, other project objectives were moved forward to ensure efficient use of laboratories and staff time. Consequently, CyHV2 was detected at a large goldfish farm in Victoria on two occasions and at a smaller farm on the NSW Central Coast. In the same period, the virus was also detected in wild goldfish populations in the ACT. These results are further discussed below. There was no longer a need to demonstrate whether fish infected with the virus were passing through quarantine undetected. CyHV2 is now considered endemic in Australia and the need for imported goldfish to be declared free of the viral infection is no longer required by AQIS (Appendix 5; BAA 2011/16).

For Survey 2b, three consignments of goldfish were received from wholesalers consisting of domestically produced and imported fish (Table 9). CyHV2 was detected in all three consignments. The prevalence of CyHV2 detection was 6% (1.6-16.5; 95% CI) in the goldfish that recently completed quarantine and were ready for dispersal to retail outlets (Table 9).

Four goldfish farms participated in Survey 4 for the detection of CyHV2 in domestic production. CyHV2 was repeatedly detected at two farms located in Victoria (Farm 1) and the Central Coast, NSW (Farm 3) and was not detected at two others (Farm 2 and 4). Initially, CyHV2 was detected at Farm 1 located in Victoria (Table 10; Figure 7, 8). This confirmed that CyHV2 was endemic in Australia in one very large goldfish farm (Figure 9). Farm 1 has traded for several decades and is considered to be an open system with respect to movement of fish and it distributes fish widely in Australia. The prevalence of CyHV2 at the Victorian farm was 2.7% from two seasonal samples. For Farm 3 based at the NSW Central Coast, the prevalence of CyHV2 detection at the first sample point was 6%. However the prevalence was much higher (33-38%) from the subsequent samplings. The owner of Farm 3 reported that goldfish from Farm 1 were brought to the farm between the first and second sampling period.

Farm 2 located in northern NSW was considered to be free of CyHV2. The virus was not detected in four seasonal samples collected during 2010-2011. The owner reported that the farm was closed to the importation of goldfish. Also, CyHV2 was not detected in the single sample of goldfish collected from

Farm 4 (Table 10). Several attempts were made to complete subsequent sampling at Farm 4. However, the farm declined further involvement with the project.

Wild goldfish were collected from peri-urban and rural areas from the ACT and Victoria and submitted for CyHV2 testing (Survey 6). CyHV2 was detected in goldfish caught in Cotter Reservoir in October 2010 and again during March 2011 (Table 11; Figure 10). Also, CyHV2 was detected in goldfish caught in Victoria on two samplings from the Ovens River and one sample from the Murray River (Table 11; Figure 10). Remarkably for wild populations, the detection prevalence ranged from 6-7% in the ACT to 37% in the Ovens River.

Ten consignments of goldfish were opportunistically collected from retail outlets during the project (Survey 7). CyHV2 was detected in six of these consignments with prevalence ranging from 100% to 50% (Table 12). These results were consistent with the findings from FRDC 2007/007 Aquatic Animal Health Subprogram: optimisation of PCR tests for diagnosis of megalocytivirus (gourami iridovirus) and cyprinid herpesvirus 2 (goldfish herpesvirus).

Table 9. Summary of results for goldfish (*Carassius auratus*) that were purchased from wholesalers following quarantine (Survey 2b).

| Wholesaler ID[1] (USYD lab accession) | Date received at USYD | Location of premise | Origin of fish[2] | Mean weight ± SE (g) | Mean total length ± SE (mm) | Proportion of qPCR positive fish | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|---|---|
| 1 (10/131) | July 2010 | North Sydney | Domestic | 5.0 ± 0.1 | 75.5 ± 0.7 | 1/38 pools (n=152)[3] | 0.066 (0.02-3.65) |
| 2 (10/139) | July 2010 | QLD | Domestic | 5.5 ± 0.2 | 75.1 ± 0.8 | 9/180 | 5 (2.3-9.3) |
| 5 (10/143) | July 2010 | North Sydney | Singapore | 4.42 ± 0.1 | 48.3 ± 0.5 | 3/50 | 6 (1.6-16.5) |

[1] wholesaler ID numbers correspond and continue from Table 5
[2] origin was provided by the wholesaler and could not be verified
[3] prevalence was calculated using EpiTools calculator for fixed pool size and assuming perfect tests with the total number tested in brackets

Table 10. Summary of goldfish (*Carassius auratus*) collected from domestic aquaculture producers that were submitted for CyHV2 testing (Survey 4).

| Farm ID and Location[1] | USYD Lab Accession | Date Collected | Mean weight ± SE (g) | Mean total length ± SE (mm) | Proportion of qPCR positive fish | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|---|
| 1 Victoria | 10/125 | June 2010 | 8.3 ± 0.8 | 69.2 ± 2.1 | 4/150 | 2.7 (0.73-6.7) |
| | 11/124 | May 2011 | 10.2 ± 1.2 | 68.7 ± 2.7 | 4/150 | 2.7 (0.73-6.7) |
| 2 Northern NSW | 10/109 | May 2010 | 6.6 ± 1.0 | 62.3 ± 1.9 | 0/151 | 0 (0-2.4) |
| | 10/165 | August 2010 | 4.1 ± 0.7[2] | 67.5 ± 4.6[2] | 0/158 | 0 (0-2.3) |
| | 11/005 | January 2011 | 1.6 ± 0.1 | 48.7 ± 0.9 | 0/150 | 0 (0-2.4) |
| | 11/189 | June 2011 | 5.0 ± 0.7 | 63.3 ± 1.5 | 0/150 | 0 (0-2.4) |
| 3 Central Coast NSW | 10/213 | October 2010 | 3.4 ± 0.4 | 51.8 ± 2.1 | 10/167 | 6.0 (2.9-10.7) |
| | 11/123 | April 2011 | 3.0 ± 0.1 | 53.2 ± 0.8 | 86/224 | 38 (32-45.1) |
| | 12/032 | February 2012 | 4.7 ± 0.2 | 63.9 ± 1.0 | 50/150 | 33 (25.9-41.5) |
| 4 South East QLD | 11/190 | June 2011 | 3.7 ± 0.3 | 50.3 ± 1.7 | 0/124 | 0 (0-2.9) |

[1] farm ID numbers correspond and continue from Table 6
[2] weight and length measurements were recorded for first 15 fish sampled

Table 11. Summary of goldfish (*Carassius auratus*) collected from wild populations that were submitted for CyHV2 testing (Survey 6).

| Location | USYD lab accession | Latitude and longitude coordinates | Date collected | Mean weight ± SE (g) | Mean total length ± SE (mm) | Proportion of qPCR positive fish | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|---|---|
| Cotter Reservoir ACT | 11/120 | -35.3179, 148.9284 | 27-28 October 2010 | 29.1 ± 3.1 | 79.8 ± 3.2 | 9/146 | 6.2 (2.9-11.4) |
| | | | 1-2 March 2011 | 26.4 ± 2.4 | 105.3 ± 2.8 | 14/198 | 7.1 (3.9-11.6) |
| Ovens River, VIC | 11/205 | -36.4100, 146.4502 | January 2011 | 148 ± 19.8 | 207.3 ± 15.0 | 0/8 | 0 (0-36.9) |
| | | -36.5020, 146.6047 | April 2011 | 38.9 ± 9.7 | 110.9 ± 9.1 | 15/41 | 36.6 (22.1-53.1) |
| | | -36.0893, 146.2191 | October 2011 | 65.2 ± 8.0 | 147.8 ± 7.5 | 1/15 | 6.7 (1.7-31.9) |
| Murray River, VIC | 11/205 | -35.9675, 145.8483 | June 2011 | 26.7 ± 5.4 | 87.9 ± 3.0 | 8/75 | 10.7 (4.7-19.9) |
| Dartmouth, 8 Mile Creek, VIC | 11/205 | -36.6042, 147.5409 | Feb 2011 | 17.5 ± 3.0 | 100.3 ± 4.8 | 0/4 | 0 (0-60.2) |
| Nagambie/Murchison Region, VIC | 11/205 | -36.665, 145.1724 | Feb 2011 | 9.9 ± 3.2 | 67.8 ± 7.0 | 0/19 | 0 (0-17.6) |
| Hughes Creek, VIC | 11/205 | -36.9080, 145.2345 | March 2011 | 33.4 ± 10.4 | 121.0 ± 10.3 | 0/5 | 0 (0-52.2) |
| Lance Creek, VIC | 11/205 | -38.5176, 145.6774 | April 2011 | 24.0 ± 3.4 | 110.3 ± 5.1 | 0/7 | 0 (0-41.0) |
| Buffalo River, VIC | 11/205 | -36.5545, 146.6860 | April 2011 | 22.9 ± 1.9 | 105.0 ± 5.0 | 0/2 | 0 (0-84.1) |
| VIC | 11/205 | unknown | unknown | 120.0 ± 13.5 | 182.0 ± 8.4 | 0/13 | 0 (0-24.7) |

Table 12. Summary of goldfish (*Carassius auratus*) collected from retail outlets for CyHV2 testing (Survey 7).

| USYD Lab Accession | Date Collected | Location of retailer | Origin of fish[1] | Proportion of PCR positive fish | Percent positive (95% confidence interval) |
|---|---|---|---|---|---|
| 10/156 | 30 July 2010 | Northern NSW | Melbourne | 30/30 | 100 (88.4-100) |
| 10/157 | 13 August 2010 | Northern NSW | Melbourne | 29/29 | 100 (88.1-100) |
| 10/158 | 30 July 2010 | Northern NSW | Melbourne | 29/30 | 96.7 (82.8-99.9) |
| 10/163 | 5 July 2010 | Northern NSW | Unknown | 3/5 | 60 (14.7-94.7) |
| 10/159 | 16 July 2010 | Northern NSW | Melbourne and Brisbane | 1/2 | 50 (1.2-98.7) |
| 11/012 | 4 November 2010 | Northern NSW | Domestic | 1/2 | 50 (1.2-98.7) |
| 10/160 | 30 July 2010 | Northern NSW | Unknown | 0/9 | 0 (0-33.6) |
| 10/162 | 13 August 2010 | Northern NSW | Unknown | 0/1 | 0 (0-97.5) |
| 11/007 | 4 November 2010 | Northern NSW | Melbourne | 0/1 | 0 (0-97.5) |
| 11/009 | 4 November 2010 | Northern NSW | Melbourne | 0/1 | 0 (0-97.5) |

[1] origin was provided by the retail outlet owner or manager and could not be verified

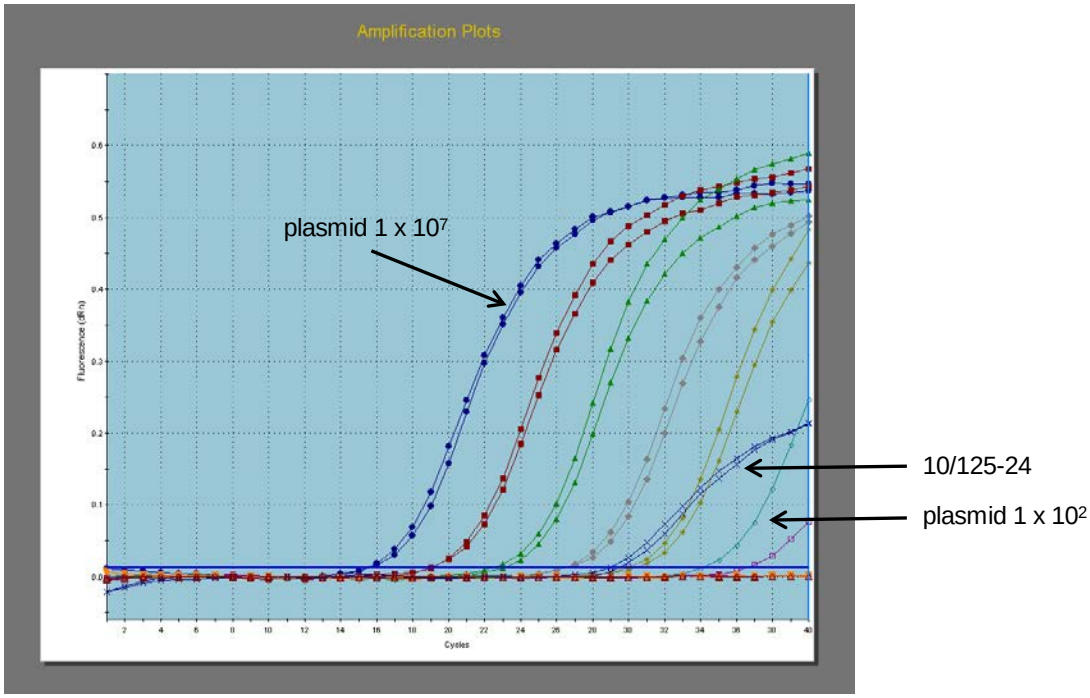


Figure 7. Amplification curves for the plasmid control *pCyHV-2*-DNApol (serial dilution) and a CyHV2 positive sample (SVC 10/125-24) from the survey of domestic farms.

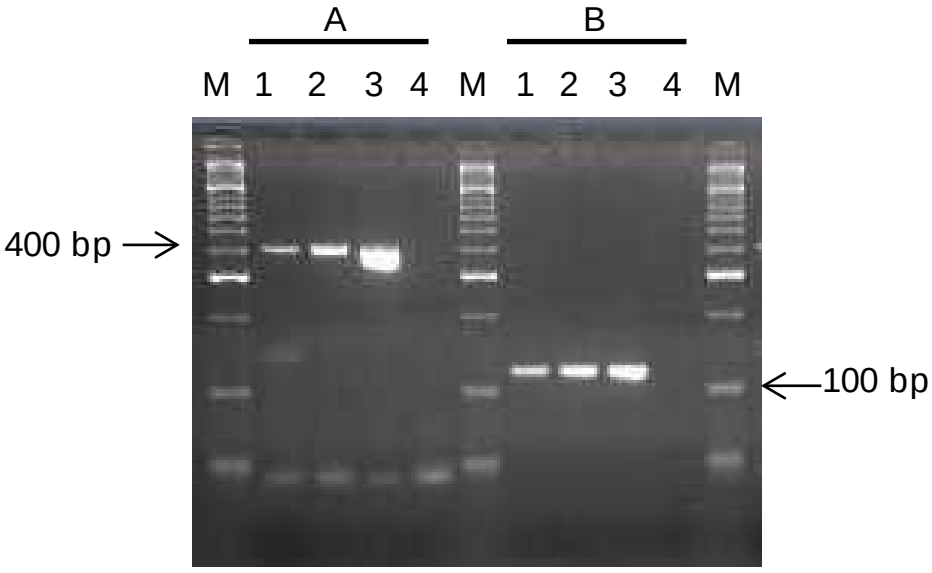


Figure 8. Agarose gel electrophoresis of CyHV2 DNA in tissues of farmed goldfish (SVC 10/125-24), (1), molecular weight marker (M), control DNA (2), pCyHV-2-DNApol plasmid DNA (3) and no template control (4). Amplicons are of (A) 401 base pairs (bp) and (B) 121 bp for the DNApol gene (See Appendix 3 for primer details).


Figure 9. Nucleotide sequence alignment for the partial DNA polymerase gene for CyHV2 (GenBank: DQ085628.1) compared with a positive goldfish from a domestic farm (10/025-24). Primer pair sequences are shown in colour with name and direction indicated above.

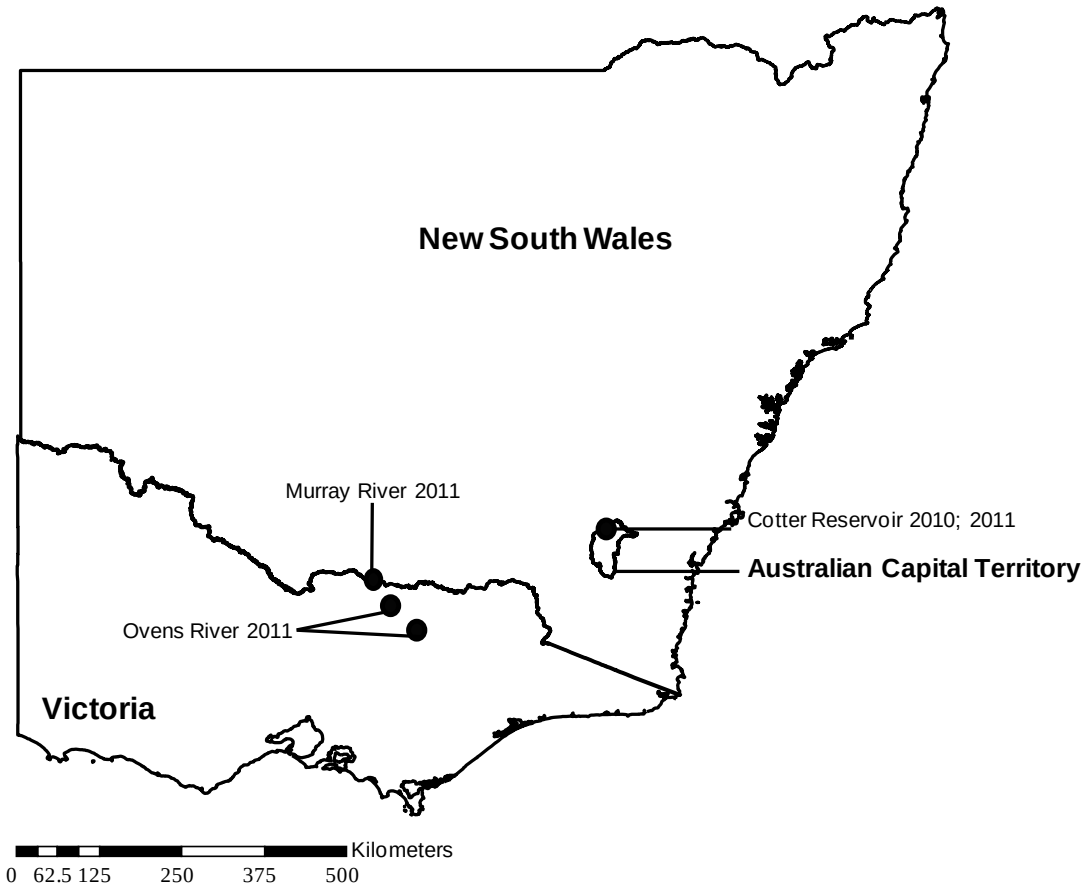


Figure 10. Sites of collection of wild goldfish (*Carassius auratus*) in 2010 and 2011 with positive detection of CyHV2.

**Objective 7: To determine whether domestic goldfish free of CyHV2 succumb to disease when cohabitated with imported goldfish carrying CyHV2**

As described above, it was reported anecdotally that goldfish from a farm in northern NSW experienced high levels of mortality once they entered the retail environment. It was suggested that the cause of mortality was related to CyHV2. The objective of the cohabitation trials was to determine whether domestic goldfish free of CyHV2 succumb to disease when cohabitated with goldfish carrying CyHV2.

To complete this objective the research steps were:

i. provide tissues containing CyHV2 for use in the *in vivo* amplification of the virus
ii. *in vivo* amplification of CyHV2 to generate a volume of homogenous pathogenic inoculum for transmission study
iii. complete transmission trials to compare the susceptibility of goldfish from a naïve and an endemic farm to CyHV2 via cohabitation exposure

**i: Identify and verify tissues containing CyHV2**

From the archive of CyHV2 positive goldfish, we selected one submission of goldfish from the retail survey which demonstrated a high prevalence and a high viral load during the original testing in 2010 (USYD lab accession 10/156, Table 12). Quantification of viral DNA from the tissue homogenates was calculated as the copy number per 5 µl of template DNA and interpolated from the plasmid DNA standard curve, with a reaction efficiency of 90 to 110%. Moreover, sequence data were obtained from this submission and the virus in fish tissues was confirmed to be CyHV2. At the time of submission, the retailer had reported dead and moribund goldfish. The DNA was re-extracted from the tissue homogenates (1:10 filtered tissue homogenate with antibiotics) and re-tested for CyHV2 from the 10 individual goldfish with the highest copy number in 2010. Following this, the tissue homogenate from the five goldfish with the highest copy number from the second extraction were pooled and retested for CyHV2 (Table 13). This pooled tissue homogenate had a copy number equal to $1 \times 10^7$ and was used as the inoculum for the *in vivo* amplification of CyHV2 (see Trial 1 below).

Table 13. Quantification of CyHV2 in moribund goldfish from a retail outlet selected for use in the *in vivo* amplification trial. Values represent an average of duplicate reactions.

| UYSD lab accession no. | Ct value (original) | Viral load expressed as copy number (original) | Ct value ($2^{nd}$ extraction) | Viral load expressed as copy number ($2^{nd}$ extraction) |
|---|---|---|---|---|
| 10/156-7 | 16.16 | $4.34 \times 10^7$ | 16.44 | $2.58 \times 10^7$ |
| 10/156-10 | 14.44 | $1.26 \times 10^8$ | 16.67 | $2.21 \times 10^7$ |
| 10/156-29 | 16.46 | $3.57 \times 10^7$ | 17.29 | $1.50 \times 10^7$ |
| 10/156-12 | 15.98 | $4.79 \times 10^7$ | 17.52 | $1.39 \times 10^7$ |
| 10/156-26 | 17.14 | $1.17 \times 10^7$ | 17.59 | $1.25 \times 10^7$ |

**ii: *in vivo* amplification of CyHV-2**

*Trial 1: Archived tissue homogenate from moribund/dead goldfish collected from a retail outlet in 2010*

Next, it was necessary to generate a volume of CyHV2 infective tissue to serve as the challenge inoculum for the transmission trials. Eight goldfish (10 cm standard length ca.) were obtained from a hatchery in northern NSW with no previous history of CyHV2 (see Table 10). The fish were housed in a glass aquarium (120 L) with an established biological filter and maintained at 22°C. Each fish was anaesthetised, weighed, measured and given an intraperitoneal injection of 100 µL of the pooled tissue homogenate (from Table 13). Fish were inspected once to twice daily for signs of morbidity and maintained for up to 28 days.

No mortalities occurred during the 28 day observation period. On day 28 post injection (PI), fish were euthanized and sampled. All eight goldfish were negative for CyHV2 by qPCR. The inoculum used in this trial was from goldfish collected from a retail outlet, where the precise handling of the dead and moribund fish was unknown. It was speculated that this could have led to inactivation of virus.

*Trial 2: Archived tissue homogenate from apparently healthy goldfish collected in 2012 from a NSW farm*

Due to the lack of evidence of CyHV2 infection in Trial 1, we selected a 2012 positive laboratory accession (SVC 12/032) from a targeted sampling day at a goldfish farm for *in vivo* amplification. As above, homogenates with the highest copy numbers were pooled, and re-tested by qPCR (Table 14). As above, eight goldfish from a farm with no history of CyHV2 were held and injected with 100 µL of inoculum. No mortalities occurred during the 28 day observation period. On day 28 PI, fish were euthanized and sampled for CyHV2. All eight goldfish were negative for CyHV2 by qPCR.

Table 14. Quantification of CyHV2 in apparently healthy goldfish from a domestic farm selected for use in the second *in vivo* amplification trial. Values represent an average of duplicate reactions.

| USYD lab accession no. | Ct value (original) | Viral load expressed as copy number (original) | Ct value of pooled inoculum | Viral load expressed as copy number |
|---|---|---|---|---|
| 12/032-70 | 24.50 | $3.80 \times 10^4$ | | |
| 12/032-72 | 22.56 | $1.40 \times 10^5$ | | |
| 12/032-75 | 25.94 | $1.43 \times 10^4$ | 29.63 | $1.20 \times 10^4$ |
| 12/032-77 | 25.68 | $1.70 \times 10^4$ | | |
| 12/032-95 | 25.14 | $3.90 \times 10^4$ | | |

*Trial 3: Effect of a temperature stressor on inducing CyHV2 in goldfish*

Interestingly, apparently healthy and moribund goldfish have been reported to have similar viral densities with up to $10^7$ copies per µg DNA (Goodwin et al. 2009). Clinical outbreaks of CyHV2 have been associated with sudden and severe drops in water temperature of at least 10°C, such as those experienced by goldfish during times of transportation between farms and retail outlets (Goodwin et al., 2009). The objective of this trial was to induce clinical disease in goldfish from a farm with a history of CyHV2 by exposing them to a cold shock following an IP-injection with the virus or by cohabitation.

A group of 120 goldfish representing two different strains (Strain A = 40 and Strain = 80) were obtained from a farm in NSW with a history of CyHV2 (Table 10). The farm owner reported that the fish were collected from ponds with water temperatures around 15°C. Upon arrival at Camden, 10 fish from each strain (A and B) were randomly selected and tested for CyHV2. Two fish from Strain A were positive for CyHV2 detection by qPCR. In separate tanks for each strain, half of the fish were held at 18°C to serve as a control group and the other half were held at 28°C for 7 days. Five fish from each of the 28°C tanks were injected with 100 µL of inoculum (same homogenate as Trial 1). Following the injection, all fish (injected and tank-mates) held at 28°C were immediately transferred to an identical tank held at 18°C. The control groups and the treatment tanks consisting of injected and cohabitating fish were observed for clinical signs, morbidity and mortality for 60 days.

No mortality was observed in any of the tanks. After 60 days, all fish were euthanized and sampled for CyHV2 testing. From the cold shock group for Strain A, 2 of the 25 cohabitating goldfish were positive for CyHV2 by qPCR. As these fish were from a farm with a previous history of Cy-HV2, they may have been sub-clinical carriers prior to experimentation. All other treated and control fish were negative. The cold shock did not induce disease or lead to the development of a sub-clinical carrier state following IP-injection or cohabitation exposure to CyHV2.

### iii: Complete transmission trials to compare the susceptibility of goldfish from a naïve and an endemic farm to CyHV2 via cohabitation exposure

*Trial 4: Cohabitation experiment to mimic retail outlets*

The original anecdotal observation arose from the mixing of domestically farmed goldfish with those from imported sources at retail outlets. We purchased 20 goldfish consisting of 10 comets and 10 fantails from a retail outlet in Campbelltown, NSW and one moribund goldfish (comet) was collected at the same time. The following day, five fish from each variety were randomly selected and tested by qPCR for CyHV2. Two of the fantail goldfish and the moribund comet goldfish were positive for CyHV2. An additional 12 comets and 11 fantails were collected (48 hours after the original purchase) from the same tanks. The store manger had reported that no new fish had been received since the original collection.

A total of 17 comet and 16 fantail goldfish from the retail outlet were separately cohabitated (held as described in Trial 1) with each of 10 goldfish sourced from a farm with no history of CyHV2. Goldfish were visually distinguished from the farmed fish based on their colour and fin morphology. As a final attempt to induce a clinical infection, 10 naive goldfish were each injected with 70 µL of pooled homogenate from the three CyHV2 positive fish (2 healthy and one moribund) collected from the retail outlet (Table 15). Fish were observed daily to twice daily for signs of morbidity and mortality for 60 days. All surviving fish were sampled on the final day for qPCR testing for CyHV2 detection.

Table 15. Quantification of CyHV2 in goldfish from a retail outlet selected for use in the *in vivo* amplification in Trial 4. Values represent an average of duplicate reactions.

| USYD lab accession no. | Ct value | Viral load expressed as copy number |
|---|---|---|
| 12/135-1 (moribund) | 34.16 | $9.68 \times 10^2$ |
| 12/135-9 | 33.07 | $2.09 \times 10^3$ |
| 12/135-11 | 33.65 | $1.44 \times 10^3$ |

A total of 19 goldfish died during the observation period with three fish from the retail outlet testing positive for CyHV2 (Table 16). The other fish were presumed to have died from other causes, although no other diagnostic procedures were undertaken. The virus was detected in two goldfish from the retail outlet that were sampled on Day 60. All of the cohabitating farm-sourced goldfish that either died or were sampled on the final day were negative for CyHV2 by qPCR. Furthermore, none of the injected goldfish from the farm with no history of CyHV2 died during the 60 day observation period and CyHV2 was not detected in any of these fish.

Our results did not confirm the anecdotal observation of farmed fish succumbing to CyHV2 associated disease when in contact with goldfish from other sources. As CyHV2 was the only pathogen we sought to confirm using an objective test, we cannot rule out the presence of other pathogens which might be transferred between fish from the different sources.

Table 16. Summary of the number of CyHV2 positive goldfish following cohabitation of goldfish from a domestic farm and a retail outlet (Trial 4).

| Tank No. | Goldfish variety (source) | Proportion died | No. died that were CyHV2 positive | Proportion alive on day 60 | No. alive that were CyHV2 positive |
|---|---|---|---|---|---|
| Tank 1 | Comet (retail) | 8/17 | 2 | 9/17 | 2 |
| | Comet (farm) | 2/10 | 0 | 8/10 | 0 |
| Tank 2 | Fantail (retail) | 6/16 | 1 | 11/16 | 0 |
| | Comet (farm)* | 3/8 | 0 | 5/8 | 0 |

* qPCR testing was not completed on two fish due to non-viable tissue samples

**Objective 8: To extend the findings of this study to the ornamental fish sector in Australia to provide information for use by DAFF.**

The project team provided advice to DAFF as requested. Milestone Reports were provided to DAFF Animal Biosecurity – Aquatic Animals (Dr Geoff Grossel) during the project. As highlighted above, importation conditions for goldfish with respect to freedom of CyHV2 were removed in 2011 based on the findings of this project. Throughout the project, consultative discussions were held with staff from DAFF at their request with the research leaders (Becker and Whittington). Also, Professor Richard Whittington has provided informal advice to DAFF on several occasions regarding testing procedures to detect ornamental fish viruses, in particular those belonging to the genus *Megalocytivirus*. The project team members Becker, Whittington, Tweedie and Landos provided advice to assist staff at Queensland DAFF following the detection and notification of megalocytivirus in the farmed platy. Laboratory reports with results were made available to the ornamental fish farms.

See Appendix 4 for a list of publications relating to this project.

## Overall final discussion

*Objective 1: To determine whether DGIV is entering Australia despite quarantine practices*

Findings from Survey 1a revealed that five species of gourami commonly exported from South-East Asia and South Asia are arriving at Australia's border with megalocytivirus infections. Sequence information provided confirmation that the isolates were ISKNV-like megalocytivirus. This indicated that the pre-importation conditions were not sufficient to detect fish with DGIV infections and prevent them from arriving at Australia's border.

Findings from Survey 1c indicated that sick and moribund fish with megalocytivirus infections were being identified during the quarantine period by AQIS. However, detection of DGIV infections in post-quarantine fish from wholesalers (Survey 2) mirrored the results from the pre-quarantine samples. Megalocytivirus infections were found in five of six consignments of fish from wholesalers. This suggested that quarantine was ineffective at detecting and removing DGIV infected fish. Six species of gourami were positive for DGIV with species belonging to the Genus *Trichogaster* having consistently high prevalence (e.g. 15-30% positive).

*Objective 2: To determine whether CyHV2 is entering Australia despite quarantine practices*

During the early stages of the project, CyHV2 was identified in goldfish from two domestic producers and from wild populations collected in the ACT. Presumably, goldfish with CyHV2 infections have passed quarantine and entered the domestic goldfish population and the virus has spread through live fish trading. Based on these findings, CyHV2 is now considered endemic in Australia (Survey 1b).

*Objective 3: To determine whether DGIV is already established in farmed gourami in Australia*

Finding ornamental fish producers was a challenge throughout the project for Survey 3. Ornamental fish from three farms were tested for the presence of DGIV. The virus was found in platy from one farm located in Queensland. Sequence information provided confirmation that the isolates were ISKNV-like megalocytivirus. This farm did not possess an aquaculture permit and would have continued trading in this manner until the detection of a notifiable disease. DGIV was not found in guppies from the two other farms.

*Objective 4. To determine whether CyHV2 is already established in farmed goldfish in Australia*

A total of four farms supplied goldfish for CyHV2 testing and the virus was repeatedly found at two farms. The virus is considered endemic at these two farms. One farm is considered the largest producer and distributor of goldfish

in Australia and the other is smaller with most of their fish going to Sydney-based retail outlets. During the project, only one farm in northern NSW was considered free of CyHV2 with negative results in all four seasonal samples. A single sample of goldfish was negative from the farm in Queensland, however additional testing is needed in order establish if this farm is free of CyHV2.

*Objective 5: To determine whether DGIV is already established in wild gourami in Australia*

Wild populations of Blue/gold gourami were located east-southeast of Townsville, Queensland and two seasonal samples were collected and tested. All samples were negative and these wild populations were considered to be free of DGIV infections.

*Objective 6: To determine whether CyHV2 is already established in wild goldfish in Australia*

CyHV2 was detected and confirmed with sequence data from goldfish caught in Cotter Reservoir in October 2010 and March 2011. The virus is considered endemic in this population. Moreover, the virus was detected in populations of wild goldfish collected from the Ovens and Murray Rivers in Victoria. Fish from the Murray River were collected downstream of Yarrawonga Weir. It would be prudent to assume that this population of goldfish to the next physical impediment (e.g. Torrumbarry Weir) are endemically infected with CyHV2 infections. The same assumption should be made of goldfish populations in the Ovens River.

*Objective 7: To determine whether domestic goldfish free of CyHV2 succumb to disease when cohabitated with imported goldfish carrying CyHV2*

Four transmission trials were completed using a combination of intraperitoneal injection and cohabitation to induce herpesviral haematopoietic necrosis in goldfish from farms with and without a history of CyHV2. Naive goldfish were injected with a pooled inoculum that was obtained from fish that were qPCR positive with high viral loads ($>10^6$ copies) and that were either moribund/dead or apparently healthy. We were unable to induce clinical disease or infections associated with CyHV2 using injection or cohabitation transmission models with or without temperature shock. These results did not confirm the anecdotal observation of farmed fish succumbing to CyHV2 associated disease when in contact with goldfish from other sources.

*Objective 8: To extend the findings of this study to the ornamental fish sector in Australia and provide information for use by DAFF*

Biannual milestone reports containing the research findings from the project were made available to DAFF and the project team provided advice to DAFF as requested. Midway through the project, findings from Survey 4 and 6 were used by DAFF to support a policy change regarding the importation conditions for goldfish. Laboratory reports were available to fish farms upon request.

*Incursions of DGIV and CyHV2 in Australia*

In Australia, around 19 million ornamental fish are imported each year (Biosecurity Australia, 2010), representing an efficient system for the translocation of ornamental fish pathogens. Australia is one of a handful of countries in the world with stringent import controls for ornamental fish (Whittington and Chong, 2007). In particular, Australia's controls include health certification at export premises and upon arrival, a visual fish health inspection and a quarantine period of 7 to 21 days depending on species (Appendix 5). Despite quarantine regulations during importation, there have been several incidents in Australia where exotic pathogens such as DGIV and CyHV2 have affected farmed or free-living fish species.

Planning for the current project began following mortality events at fish farms that were linked to exotic viruses. A significant iridovirus epidemic at a Murray cod farm in Victoria occurred in 2003. Over the course of a few weeks, approximately 90% or 9000 Murray cod fingerlings died. At the time, the unidentified iridovirus was considered to be exotic to Australia (Lancaster et al., 2003). Molecular studies by Go et al. (2006) demonstrated that the iridovirus from the outbreak shared a greater than 99% sequence homology with dwarf gourami iridovirus isolated from dwarf gourami purchased from Sydney aquarium shops. At about the same time in 2003, mortality events at a goldfish farm in Western Australia were caused by infections with goldfish haematopoietic necrosis virus (or CyHV2) (Stephens et al., 2004). It was suggested that sub-clinically infected goldfish were passing quarantine regulations and coming into contact with domestic stocks through live fish trading.

Due to the risk to aquaculture, recreational fisheries and biodiversity, there was an urgent need to develop and validate diagnostic tests for both DGIV and CyHV2. As such, under FRDC 2007/007 Aquatic Animal Health Subprogram: optimisation of PCR tests for diagnosis of megalocytivirus (gourami iridovirus) and cyprinid herpesvirus 2 (goldfish herpesvirus), conventional and quantitative PCR assays were developed and transferred to diagnostic laboratories around Australia to detect these viruses and distinguish them from other exotic but closely related viruses such as red sea bream iridovirus and other cyprinid herpesviruses (e.g. CyHV1 and CyHV3), respectively. Also included in the project was an opportunistic survey of sick or moribund fish collected from retail outlets in Sydney as a way of obtaining sample material to test the new PCR assays. The survey revealed CyHV2 in 17% of goldfish. Also, DGIV was detected in several species including: Dwarf gourami, Blue/gold gourami, Oscar (*Astronotus ocellatu*s) and Cichlid. However, since retail outlets contained an assortment of imported and domestic fish sharing the same environment, the source of the viruses could not be identified. Furthermore, domestic goldfish breeders have anecdotally claimed that their stock succumb to diseases when brought into contact with imported goldfish in wholesale and retail premises. From these findings, the logical progression was to ascertain if DGIV and CyHV2 were indeed passing through quarantine undetected and if so, to determine if the viruses have become established in domestic stocks. This was achieved through the

current project, FDRC 2009/044 Aquatic Animal Subprogram: surveys of ornamental fish for pathogens of quarantine significance. A series of surveys were conducted targeting imported and domestic stocks of ornamental fish to detect DGIV and CyHV2.

From the current project, DGIV was consistently found in several species of gourami imported from six different countries. The virus was also found in stocks of gourami from wholesale premises, at retail outlets and one domestic fish farm. The findings indicate that the health certification at exporting countries was insufficient to detect and prevent fish with DGIV being exported to Australia. Once fish arrive in Australia, quarantine and visual inspection were insufficient to identify fish with DGIV infections. This was evidenced by the fact that five of six consignments of imported gouramis that had recently passed quarantine had detectable levels of DGIV. Finally, DGIV was found in a group of platy at a domestic ornamental fish farm. At the time of collection, there was no reported outbreak and the infection was presumably sub-clinical. Fortunately, the farm operates at two separate locations with many species of gourami raised at a location separate to the infected platy. Biosecurity Queensland is aware of the situation and is working with the owners to resolve it. No directives have been issued from the Queensland CVO regarding the presence of DGIV at the farm.

This project supports the recommendation that laboratory testing be carried out as an effective way of detecting DGIV in imported ornamental fish (Whittington and Chong, 2007; Biosecurity Australia, 2010). The detection of DGIV at a domestic farm is concerning due to the risk of spreading (and potentially amplifying) the virus through the live fish trade to other farms and retail outlets and the risk of releasing the virus into natural waterways through contaminated effluent and other waste. The lack of a plan to deal with such an incursion of an exotic pathogen is concerning. Both of these pathways increase the opportunity for DGIV to become established in wild populations, which would impact on recreational fisheries, biodiversity and aquaculture development. As shown during the 2003 outbreak, Murray cod are highly susceptible to DGIV. As the virus has a wide host range, other species of native fish should be considered at risk to infection, in particular the Australian freshwater cods and perches belonging to the Genera *Maccullochella* and *Macquaria*. However, until further research is completed, all 22 species in the Family Percichthyidae should be considered potentially susceptible.

The results for the CyHV2 surveys were similar to DGIV, in that the virus was found at wholesaler premises and farms, but notably also in several populations of wild goldfish. The virus was found in feral populations of goldfish in the ACT and more densely populated rural areas of Victoria. It is presumed to have originated from infected stocks that were imported to Australia. There are no goldfish farms upstream of Cotter Reservoir (or anywhere near the ACT) and there is no mechanism for natural dispersal of fish from downstream areas as the dam wall is a complete barrier to fish passage. One explanation for the presence of the virus is that unwanted goldfish with sub-clinical infections were released by pet owners into natural waterways. Existing feral populations of goldfish were then exposed and the

virus was able to persist. Another possibility is that infected fish were transferred to domestic farms, where the virus was able to be amplified in a naïve population and released through effluent and live fish. As goldfish were first introduced to the Canberra region in the 1880s, the virus may have been here for a very long time.

The findings of the project were a demonstration that CyHV2 is established in Australia and were used to inform quarantine policy. Within nine months of the confirmation of CyHV2 being endemic at domestic farms, AQIS revoked the requirement for goldfish exported to Australia to be certified free of CyHV2 (Appendix 6; BAA 2011/16). This is a clear demonstration of an aquatic pathogen from the ornamental fish industry with quarantine significance can become established in farmed and wild populations. Also, it provides evidence for a pathway for exotic pathogens to become established in wild fish populations. This is of particular significance to Australia as there are many endemic and ecological sensitive populations of fish. The results of the CyHV2 surveys can be used to inform risk analysis for the importation of other ornamental fish species.

Over one billion ornamental fish, comprising of more than 4 000 freshwater and 1 400 marine species are traded internationally each year (Whittington and Chong, 2007). Although a large number of countries are involved in the industry, Asia is by far the largest exporter of ornamental fish, providing more than 55% of the world supply (Ploeg, 2007). Within Asia, species of fish originating from all over the world are bred for the commercial markets, with Singapore the leading exporter followed by Hong Kong, China, Indonesia and Malaysia (Ploeg, 2007). Singapore and Hong Kong also act as hubs, aggregating numerous species from neighbouring countries for on-sale to developed countries (Ling and Lim, 2005). In these large exporting facilities, fish are congregated from the wild, farms, wholesalers and other exporters from all over the world (Ling and Lim, 2005). Based on this structure and distribution system of ornamental fish, it is likely that DGIV and other exotic aquatic pathogens will continue to enter Australia unless quarantine practices are improved.

## BENEFITS AND ADOPTION

The benefits and beneficiaries of this research project align with those identified in the project application, which was based on consultation with Commonwealth Department of Agriculture, Fisheries and Forestry, the Murray Darling Basin Authority and the FRDC. The project is of national significance and will have a direct benefit for many stakeholders. Findings from the project were used in 2011 to support changes to national policy. Within nine months of reporting the detection of CyHV2 at domestic farms, AQIS acted to remove the import condition for goldfish to be free of CyHV2.

In 2008, Biosecurity Australia initiated an IRA to review Australia's freshwater ornamental finfish policy with respect to quarantine risks associated with DGIV and related viruses. The final policy determination is on hold awaiting the results of this project. The findings from this project relating to the detection of DGIV from imported and domestically sourced ornamental fish will be used to support the IRA and national policy decisions. Of particular importance is the accurate information concerning the occurrence of DGIV in imported ornamental fish (pre-border) prior to their contact with domestic-origin fish or at retail outlets.

The private sector has benefited by removal of unnecessary import conditions requiring health certification for freedom of CyHV2. Benefits for farms that participated included improved fish health and information on their DGIV and CyHV2 testing results for better management practices.

The public has benefited and will benefit by improved quarantine policy and improved conservation management of threatened freshwater finfish species. This will help reduce the chance of DGIV becoming established in wild populations of ornamental fish and thereby posing a risk to biodiversity.

## FURTHER DEVELOPMENT

This project was successful in providing a clear demonstration that an aquatic pathogen from the ornamental fish industry with quarantine significance can become established in farmed and wild populations. It has also shown that there is an ever present risk of exotic pathogens arriving at Australia's border.

Recommendations for further development include:

1. Revision of quarantine policy to prevent incursions of exotic viruses and other pathogens from imported ornamental fish into domestic stocks. There is clear evidence that exotic pathogens from the ornamental fish industry can cause disease and become established in farmed and wild populations of fish. This is based on our current knowledge of at least two incursions of DGIV at domestic farms and the widespread distribution of CyHV2 in goldfish populations. Also the project findings indicated that the health certification at exporting countries was insufficient to prevent fish with exotic pathogens being exported to Australia. Biosecurity Australia announced a trial of a proposed new system for managing the biosecurity risks of imported ornamental fish with an emphasis on risk management placed at exporting countries (Appendix 6; BAA 2012/23). The reliance on off-shore authorities for risk management should proceed with caution based on the project findings. The revision of policy with respect to Australia's CyHV2 status took less than one year from the initial report of the virus at domestic farms. Strengthening of policy dealing with the quarantine risks associated with DGIV and related viruses due to the threat to native freshwater fish and the aquaculture industry is recommended. Policy revision and new policy development should consider increased quarantine periods for species susceptible to DGIV, increased surveillance, mandatory diagnostic testing for rejected consignments during quarantine, mandatory reporting of mortalities during quarantine, restriction of ornamental species allowed for importation and increased tracking and traceability for the entire industry.

2. Risk analysis of the aquarium trade as a pathway for release of DGIV in Australia and the risk of exposure to native fish species. While DGIV has not yet been recorded in the wild, it is routinely detected in ornamental fish from aquatic retail outlets and the virus has been the cause of mass mortality at a Murray cod farm in 2003. From this project, DGIV was detected in platy from a farm in Queensland. In Australia, the aquarium industry is recognised as a major pathway for human-assisted dispersal of ornamental fish (Lintermans, 2004). It is recognised that 22 of the 34 alien fish species with established populations in Australian freshwaters originate from the ornamental trade (Lintermans, 2004), with the number of established exotic aquarium species continuing to grow (Lintermans 2013). Given the high prevalence of DGIV in ornamental fish in aquarium retail outlets and the existence of pathways for these ornamental fish to enter natural waters, the potential for the release of DGIV from the aquarium trade and exposure of wild fish is genuine. Further, the incursion

of CyHV2 in Australia should be considered a case study to support risk analysis.

3. Susceptibility and epidemiology of Australian freshwater fish species to DGIV. DGIV belongs to a family of viruses known as Iridoviruses. Iridoviral diseases are known to be important in aquaculture, causing significant mortality worldwide but their effects on wild populations of native fish in Australia are largely unknown. Experimental challenge trials have shown that a number of native fish, including Macquarie perch (*Macquaria australasica*) are potentially susceptible to the iridovirus, *Epizootic haematopoetic necrosis virus,* which is only found in Australia (Landgon 1989, Becker et al., 2013). As iridoviruses are characterised as having a wide host range, there is a need to determine the susceptibility of a range of native fish species to DGIV. Based on our current knowledge, it would be useful to initially focus on species closely related to Murray cod and those that commonly share waterways with them. Following, testing of wild populations of potential native and alien fish hosts is needed to determine if DGIV is in the wild. As the first outbreak of DGIV was at a Murray cod farm, there is a need to determine the occurrence of DGIV at native fish farms with an emphasis on those species susceptible to DGIV.

4. The role humans play in the dispersal of ornamental fish in natural water ways in Australia. It is unclear why the public engage in activities such as releasing unwanted pet fish and the illegal use of ornamental fish as bait, which may result in the establishment of feral species in native water ways. The current project has clearly shown that healthy looking fish could be infected with exotic pathogens and could be introduced to natural waterways with their feral host. Research from the UK has found that there was an increased chance of having feral goldfish the closer a pond is located to a road or parking lot and other ornamental fish were often found in these ponds (Copp et al., 2005). Another example is the finding of CyHV2 positive goldfish in Cotter Reservoir, which is approximately 23 km from the centre of Canberra. Little information is available on the rates and dispersal pathways and mechanisms associated with the ornamental fish introductions. Research into the dispersal pathways and mechanisms will assist in the development of education or awareness programs to discourage the practice of releasing pet fish into the wild and the illegal movements of live fish.

5. Revision of policy to protect domestic ornamental fish producers from incursions of exotic pathogens and to encourage domestic production of ornamental fish. To further the recommendations suggested above, specific policy is warranted to address the risk to the domestic production of ornamental fish. There are considerable differences in the operational practices of ornamental farms relating to the number of species farmed, the buying and selling of live fish and broodstock and the variety and number of aquaculture units (e.g. tanks and ponds) compared to farms producing food fish. Also, policy revision is needed to encourage the domestic production of ornamental fish. In addition to what is identified above, policy revision and new policy development should consider

limiting species or combination of species at farms, licensing regulations, and freedom of disease certification for key pathogens.

6. Biosecurity Operations. The example of ornamental fish from the farm in Queensland operating without an aquaculture permit being potentially infected with a notifiable agent, megalocytivirus indicated the urgency for policy revision to minimize biosecurity risks. Since the time of detection and notification on 8 May 2012, Queensland Biosecurity has been in communication with farm although no action-based directives have been ordered, which suggests a lack of willingness of competent authorities to address biosecurity risks at an operational level. The rapidity with which quarantine controls can be removed following confirmation that an agent has become endemic (e.g. CyHV2) is contrasted with the length of time required for effective policy revision to exclude pathogens of significance (e.g. the IRA for DGIV began in 2008). The uncertainty of policy revision may create opportunity for agents to become endemic, such as DGIV at the Queensland farm through a delay in effective response. If DGIV enters natural waterways, eradication would be impossible and it could potentially devastate Murray cod populations. These issues should be advanced nationally through the Subcommittee on Aquatic Animal Health (SCAAH), which is an advisory committee to Animal Health Committee (AHC).

   The main data from this project, comprising the consignment identification and the results of molecular assays are summarised in tables contained in this report. Raw data are stored at the Faculty of Veterinary Science University of Sydney in electronic form and may be accessed by contacting the author.

## PLANNED OUTCOMES

The overall planned outcome of this project was to ensure the sustainability and profitability of the aquatic industry and the health of natural resources by providing industry and governments with knowledge of the establishment of DGIV and CyHV2 in Australia. This will help to make sound and risk-based decisions for use in all jurisdictions. This was in addition to a broader responsibility towards the Australian community to ensure the sustainability of Australian aquatic natural resources.

This overall outcome was achieved through the provision of scientific evidence of the incursions of exotic viruses from ornamental fish in Australia. This will facilitate design of improved quarantine policy for live imported ornamental fish, disease prevention strategies, improved policy regarding domestic aquaculture production and facilitation of fish farms being identified as being free of these viral pathogens. This will help protect recreational fisheries through improved conservation management of threatened freshwater finfish species and help promote aquaculture.

Extension of R&D results to government and industry stakeholders was achieved through scientific articles and conference presentations (Appendix 4). Several scientific articles are currently being drafted to from this project and will be submitted to leading international journals for publication. Extension of the key outcomes of the project, namely the scientific data to support the revision of national policy to prevent the incursions of exotic viruses from the ornamental fish trade, will be ongoing through consultations with DAFF and industry.

## CONCLUSION

Australia is considered to have one of the most stringent standards for the importation of ornamental fish in the world. However, the occurrence of exotic pathogens, in particular iridoviruses, in post-quarantine populations of ornamental fish has led to a conclusion that current quarantine measures provide an unacceptably low level of protection.

The findings have delivered upon all eight objectives of the project. Specifically, as a result of this project, there is definitive evidence that an exotic viral pathogen is in fact entering Australia despite quarantine practices. DGIV was readily detected from many different species of ornamental fish at our pre-border QAP, during quarantine and following quarantine at wholesalers and retail outlets. DGIV should be considered a common pathogen at these locations. Moreover, DGIV was identified at one domestic farm in Queensland. However, it was not detected in wild populations of gourami in Queensland. If DGIV is able to be established at domestic farms, there is a significant risk to the aquaculture development and conservation of native fish species. The structure and distribution system of the ornamental fish export sector in Asia allows for the global distribution of aquatic pathogens. Under current export conditions, it is likely that DGIV will continue to enter importing countries including Australia.

As a result of this project, CyHV2 was declared an established pathogen in Australia. The virus was detected at all survey locations, most notably readily identified at domestic farms and in wild populations. In September 2011, the findings were used to inform quarantine policy to revoke the requirement for goldfish exported to Australia to be certified free of CyHV2. This policy change occurred nearly eight years after the original documented outbreak at a farm in Western Australia in 2003. CyHV2 is a clear demonstration of an aquatic pathogen from the ornamental fish industry with quarantine significance can become established in farmed and wild populations. Also, it should be used as a valuable case study to provide evidence for a pathway for exotic pathogens to become established in wild fish populations. This is of particular significance to Australia as there are many endemic and ecological sensitive populations of fish. The results of the CyHV2 surveys can be used to inform risk assessment for the importation of other ornamental fish species.

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## APPENDIX 1: INTELLECTUAL PROPERTY

This project has not developed any intellectual property that requires legal protection.

## APPENDIX 2: STAFF

Many people contributed to the research described in this report.

The core project team included Joy Becker, Rebecca Maurer, Anneke Rimmer (PhD student), Marion Saddington, Alison Tweedie and Richard Whittington from the University of Sydney, Matt Landos (Future Fisheries Veterinary Service Pty Ltd) and Mark Lintermans (University of Canberra).

The Project team would like to thank Fran Stephens, Fisheries Western Australia for supplying tissues for Survey 1c, Jarod Lyon, Joanne Kearns and fisheries biologists at the Victoria Department of Sustainability and Environment for kindly collecting wild goldfish in Victoria, Ben Broadhurst and Rhian Clear, University of Canberra for collecting wild goldfish from Cotter Reservoir in the ACT and to Malcolm Pearce, Fisheries Queensland for the wild gourami collection.

The Project Team would like to thank the wholesalers, fish farmers, retail managers and other people in the ornamental industry who kindly contributed to this project.

This report was prepared by Joy Becker using results, data and other information contributed by the team members, who also commented on a draft of the report.

## APPENDIX 3: PRIMER DETAILS FOR PCR ASSAYS

Table 1. Details of primers used in this project to detect DGIV.

| Oligonucleotide | | Product size (base pairs) | Gene | Reference |
|---|---|---|---|---|
| **Name** | **Sequence (5' – 3')** | | | |
| C50 | GTTTGATGCGATGGAGACCC | 399 | MCP[1] | Go, et al., 2006 |
| C51 | ATGCCAATCATCTTGTTGTAGCC | | | |
| C1073 | AATGCCGTGACCTACTTTGC | 167 | MCP[1] | Rimmer, et al., 2012 |
| C1074 | GATCTTAACACGCAGCCACA | | | |

[1]MCP = Major capsid protein

Table 2. Details of the primers used in this project to detect CyHV2.

| Oligonucleotide | | Product size (base pairs) | Gene | Reference |
|---|---|---|---|---|
| **Name** | **Sequence (5' – 3')** | | | |
| C1153 | GTCGGTTGGACTCGGTTTGT | 121 | DNAPol[1] | Whittington, et al., 2009 |
| C1154 | CATAGTACGCCTTGAGACTC | | | |
| C1109 | CCCAGCAACATGTGCGACGG | | | |
| C1158 | GACCAAGTAGTCAGATGCTG | 401 | | |
| C1281 | GGACTTGCGAAGAGTTTGATTTCTAC | 366 | Helicase | Waltzek, et al., 2009 |
| C1228 | CCATAGTCACCATCGTCTCATC | | | |

[1]DNAPol = DNA polymerase

## APPENDIX 4: LIST OF PUBLICATIONS

### List of publications from FRDC 2009/044 (current as of 19 June 2013)

*Peer reviewed journal articles*

Rimmer AE, Becker JA, Tweedie A, Whittington RJ. Development of a quantitative polymerase chain reaction (qPCR) assay for the detection of dwarf gourami iridovirus (DGIV) and other megalocytiviruses and comparison with the Office International des Epizooties (OIE) reference PCR protocol. Aquaculture, 2012; 358:155-163.

*Conference Proceedings*

Becker JA, Tweedie A, Rimmer A, Landos M, Lintermans M, Whittington RJ. Incursions of cyprinid herpes virus 2 in goldfish populations in Australia despite quarantine practices. *In* Proceedings of the 13th International Society of Veterinary Epidemiology and Economics meeting, Maastricht, 2012.

Rimmer AE, Whittington RJ, Tweedie A, Landos M, Lintermans M, Becker JA. Prevalence of dwarf gourami iridovirus (DGIV) in imported and domestic ornamental fish in Australia and the risk they posed to native fish. *In* Proceedings of the 13th International Society of Veterinary Epidemiology and Economics meeting, Maastricht, 2012.

Rimmer AE, Becker JA, Tweedie A, Whittington RJ. Real-time polymerase chain reaction (PCR) detection of dwarf gourami iridovirus (DGIV), an important emerging exotic viral pathogen of ornamental fish in Australia. University of Sydney, Faculty of Veterinary Science Postgraduate Research Conference, Camden, Australia, 2 - 3 November, 2011.

Rimmer AE, Becker JA, Tweedie A, Whittington RJ. Real-time polymerase chain reaction (PCR) detection of dwarf gourami iridovirus (DGIV), an important emerging exotic viral pathogen of ornamental fish in Australia. Australasian Scientific Conference on Aquatic Animal Health, Cairns, Australia, 5 – 8 July, 2011.

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Rimmer AE, Becker JA, Tweedie A, Whittington RJ. The importance of emerging exotic viral pathogens of ornamental fish in Australia. Sixth International Symposium on Aquatic Animal Health, Tampa, USA, 5-9 September 2010.

Rimmer AE, Becker JA, Tweedie A, Whittington RJ. The importance of emerging exotic viral pathogens of ornamental fish in Australia. University of Sydney, Faculty of Veterinary Science Postgraduate Research Conference, Sydney, Australia, 1 – 2 November 2010.

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## APPENDIX 5: IMPORT CONDITIONS FOR ORNAMENTAL FISH

The import conditions for the commodity “Freshwater Fish (other than Salmonidae) – Live” as of 28 Feb 2013 downloaded from the AQIS Import Conditions Database (ICON). The link is provided below.

http://www.aqis.gov.au/icon32/asp/ex_casecontent.asp?intNodeId=8872757&intCommodityId=6114

**AQIS** AUSTRALIAN QUARANTINE AND INSPECTION SERVICE
DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY

Import case details - public listing **New Search**

**Commodity:** Freshwater Fish (other than Salmonidae) - Live

**Scientific name:** *Piscine*

**Synonyms:** Show Synonyms

**Country:** All countries

**End use:** Post-entry quarantine

**Date printed:** Feb 28 2013

*The information here covers AQIS quarantine requirements only and is current on the date of transmission but may change without notice. AQIS makes no warranties or representations with respect to the accuracy or completeness of that information and will bear no liability with respect to that information. Importers must satisfy quarantine concerns and comply with quarantine conditions applicable at the time of entry. The Commonwealth through AQIS is not liable for any costs arising from or associated with decisions of importers to import based on conditions presented here which are not current at the time of importation. It is the importer's responsibility to verify the accuracy and completeness of the information at the time of importation.*

*It is the importer's responsibility to identify and to ensure it has complied with, all requirements of any other regulatory and advisory bodies prior to and after importation including the Australian Customs Service, Therapeutic Goods Administration, Department of Health and Ageing, Department of the Environment, Water, Heritage and the Arts, Australian Pesticides & Veterinary Medicines Authority and any State agencies such as Departments of Agriculture and Health and Environmental Protection authorities.*

*Importers should note that this list is not exhaustive. Importers should also note that all foods imported into Australia must comply with the provisions of the Imported Food Control Act 1992 , an Act which is administered by AQIS.*

*Notification of the import must be provided to AQIS for all imported goods other than goods imported as accompanied baggage or goods imported via the mail and not prescribed under the Customs Act 1901. Notification must be consistent with Quarantine Regulations 2000 (examples include a Quarantine Entry or a Quarantine declaration).*

Condition C8429

**Conditions of Administration**

1. An Import Permit is required and must be valid at the time the goods are imported into Australia. Import Permit applications must be sent to Animal Import Operations Branch – Department of Agriculture, Fisheries and Forestry (DAFF) Canberra, for assessment.

Animal Import Operations Branch
DAFF
GPO Box 858
Fax +61 2 6272 3110
Email animallive@daff.gov.au

Canberra 2601 Phone +61 2 6272 4454

2. Import Permits are granted for a one-year period, starting from the date of issue. Importers may use the permit to import unlimited numbers of fish during this period (subject to meeting the conditions of the import permit). It is the importers responsibility to ensure that they have a current permit prior to the import of fish.

3. Importers are required to identify that they have access to a Quarantine Approved Premises for the post arrival quarantine of fish before a permit application will be approved. Quarantine premises will only be approved, as a place for performance of quarantine of live fish under section 46A of the *Quarantine Act 1908*, when they meet DAFF standards. An application form for approval of premises for the quarantine of live ornamental finfish may be obtained from DAFF.

4. DAFF must be given at least three (3) working days notice of the fish arriving in Australia. This should be done by emailing/faxing the DAFF office located in the state in which the fish will arrive in Australia. A consignment notification form is included with a DAFF Import Permit.

| | **Email/ Fax** | **Phone** | **Your email/ fax must include:** |
|---|---|---|---|
| NSW | nswaqislivefishsection@daff.gov.au<br>02 8334 7530 | 02 8334 7543 | Valid Import Permit Number<br>Date and estimated time of arrival<br>Flight number and details<br>Exporter name and country<br>List of fish species ordered<br>No of Boxes and tails<br>Invoice/packing list (no later than morning of arrival) |
| VIC | VICAQISAquariumFish@daff.gov.au<br>03 8318 6988 | 03 8318 6793 | |
| QLD | bnelivefish@daff.gov.au<br>07 3246 8660 | 07 3246 8625 | |
| Cairns | CDO@daff.gov.au | 07 4030 7852 | |
| SA | saanimalimports@daff.gov.au<br>08 8201 6188 | 08 8201 6159 | |
| WA | WAliveanimalimports@daff.gov.au<br>08 9334 1672 | 08 9334 1522 | |

5. A Quarantine Entry must be lodged for each consignment. The inspecting DAFF officer must be advised of the entry number prior to inspection.

6. It is the importer's responsibility to identify and to ensure compliance with all requirements of any other regulatory and advisory bodies prior to and after importation. It is the importer's responsibility to arrange for any additional testing for genetic and endemic infectious diseases, or for movement of fish into certain animal health zones within Australia.

7. The importer or agent must make an appointment for inspection of the fish and documentation by DAFF officers. The importer or agent may be required to be present at this inspection. The consignment will be held by DAFF until completion of inspection. Fees are payable to DAFF for all services.

8. Consignments must be addressed and sent to DAFF at the port of arrival. Each consignment must be accompanied by a valid Import Permit (or copy) or by means to allow the identification of the Import Permit and the animal veterinary certificate as required by these conditions.

9. The importer, as listed on the Import Permit, or nominated agent, must be accessible to DAFF officers and accept responsibility for ensuring that all import conditions are met including the DAFF inspection.

10. Consignments that do not meet the DAFF import conditions will remain in quarantine control, be exported or destroyed without recompense. Any fish species not listed on the Live Ornamental Fish: Permitted Species Import List will be exported or destroyed, while prohibited material or material of quarantine concern will be seized and destroyed, all at the importer's expense.

Condition C8444

**Format of the veterinary certificate**

**1.** A veterinary certificate must accompany each consignment and must:

- be written in English, and a language understood by the Competent Authority of the country of export
- meet all requirements of the "veterinary certification" section of these conditions

**2.** The veterinary certificate must include:

- the DAFF Biosecurity import permit number
- the exporters name, address, phone number, fax number and e-mail address
- the shipping invoice number
- a list of the individual boxes destined for export and the identification numbers and any other details of each box
- a list of the scientific name/s and numbers (tails of fish) in each box destined for export.

**3.**An Official of the Competent Authority of the Government of the exporting country, having an appropriate knowledge of fish health and the export premises, must endorse (sign and stamp using a stamp of the government veterinary or agriculture administration) each page of the:

- health certificate; and
- shipping invoice or packing list or species list

DAFF will only accept copies of documents where each page bears the original signature, date and stamp of the Competent Authority.

**4.** The veterinary certification must include the following information concerning the endorsing officer

- name and title
- location at which the certification was issued
- name of the government administration
- date of certification
- phone number
- fax number
- e-mail address

Condition C9976

**Importation of live freshwater fish for ornamental purposes**

These conditions apply to the importation of certain species of freshwater ornamental (aquarium) finfish that have been assessed as suitable for importation. A list of permitted freshwater species is provided at the end of these conditions.

All fish imported under this permit are to be used for display (aquarium, ornamental) purposes only. Fish imported under this permit must not be used for non-display purposes, including:

- the feeding of aquarium fish
- the feeding of aquaculture stocks;
- for aquaculture purposes.

Use of fish imported under this permit for non-display purposes is prohibited under section 33 of Part 5 of the *Quarantine Proclamation 1998*. In determining the purpose for use of the imported fish, any statement on the permit or to a quarantine officer may be deemed to be the stated use of the goods (section 5 of the *Quarantine Proclamation 1998*).

A breach of this condition is a breach of an import permit granted under the *Quarantine Proclamation 1998* and may be an offence under section 67(5) of the *Quarantine Act 1908*.

Each consignment must only include fish that are sufficiently mature to permit accurate identification. Fish that can not be identified will be exported or destroyed at the importer's expense.

Live freshwater fish may only be imported from approved countries listed below.

| | |
|---|---|
| Belgium | China |
| Federated States of Micronesia | Fiji |
| French Polynesia | Germany |
| Hong Kong | Indonesia |
| Kenya | Malaysia |
| New Caledonia | New Zealand |
| Philippines | Saudi Arabia |
| Senegal | Seychelles |
| Singapore | Solomon Islands |
| South Africa | Sri Lanka (excluding *Carassius auratus auratus* - Goldfish) |
| Thailand | United States of America (USA) |

The fish must have resided for a minimum of 14 days immediately prior to export in an Export Premises approved by a DAFF recognised Competent Authority in one of the exporting countries listed above. Standards for Competent Authorities and Export Premises Approval are detailed below.

A list of DAFF recognised Competent Authorities is provided as part of a DAFF Import Permit.

**DAFF standards for Competent Authorities in the country of export**

1. A DAFF recognised Competent Authority of the exporting country must have in place a system for the approval of freshwater ornamental finfish export premises to ensure that such premises maintain standards required for export of freshwater ornamental finfish to Australia. The system is subject to audit by DAFF at any time. Animal Quarantine Policy Memorandum 1999/62 — *Guidelines for the approval of countries to export animals (including fish) and their products to Australia* — provides guidelines for the approval of countries to export animals and their products, including ornamental finfish, to Australia.

2. The Competent Authority of the exporting country must have the authority to suspend or withdraw export certification and/ or approval of export premises at any time if the requirements are not being met.

3. The official approving export premises must be an official of the Competent Authority of the exporting country whose duties relate to fish health and who has knowledge of the premises and its operations. The official must be satisfied the premises conform to DAFF requirements for the approval of a premises for export of live freshwater ornamental finfish to Australia that no impediment exists to the signing of such an endorsement.

**DAFF requirements for Export Premises approval**

1. Premises approved for the export of freshwater ornamental finfish to Australia, a Competent Authority must have in place a system that ensures:

· the premises are managed in an efficient, professional way under competent and experienced management. Factors to consider include maintenance of the plant and equipment in working order; maintenance of accurate records of numbers and species of stocks held; dates of arrival and sources of stocks; records of any significant mortalities; and records of clinical signs and lesions and the results of any laboratory testing and treatments;

· the premises has a system for handling fish to minimise the likelihood of disease/pest entry and spread within the premises;

· the fish being held at the premises exhibit no signs of infectious disease or pests and are sourced from populations not associated with any significant disease or pests;

· the fish are not kept in water in common with farmed food fish (fish farmed for human consumption including recreational fishing) or koi carp;

· the exporter is aware of the conditions which apply to the export of fish to Australia, including the species permitted for export to Australia at the time of export, and understands the restrictions which apply to such transactions.

**Veterinary certification requirements for the import of freshwater ornamental (aquarium) fish**

(22 August 2000)

1. Only finfish listed in the Live Ornamental Fish: Permitted Species Import list are included in this consignment, and are documented on the attached invoice.

2. The fish in the consignment have been inspected within seven (7) days prior to export and show no clinical signs of infectious disease or pests.

3. The export premises is currently approved for export to Australia as meeting standards under DAFF.

[DAFF requirements for Export Premises approval are included as part of the import permit. The address of the export premises must be included as part of the veterinary certification.]

4. All fish being held at the export premises exhibit no signs of infectious disease or pests and are sourced from populations not associated with any significant disease or pests within the 6 months prior to certification.

5. All fish in the consignment have been in premises approved for export of freshwater finfish to Australia for the 14 days prior to export.

6. The fish have not been kept in water in common with farmed food fish (fish farmed for human consumption including recreational fishing) or koi carp.

7. Adequate safeguards are in place to maintain the health status of the certified fish 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.

[Certification must be based on a system of inspection, acceptable to the Competent Authority of the country of export, for it to be satisfied that there is no significant disease or pest problem in the export premises at the time of certification.]

**Additional veterinary certification for goldfish *Carassius auratus***

1. The goldfish originate from a country, zone or export premises (the population) determined to be free from spring viraemia of carp virus (SVCV) and *Aeromonas salmonicida* (other than goldfish ulcer disease strains) based on a program of monitoring and surveillance acceptable to the Competent Authority and consistent with criteria for health certification stated in the DAFF import conditions as "Criteria on which additional veterinary certification for goldfish is based".

[If any of the fish have been imported, the country/ies of origin must be listed as part of the veterinary certification. Health certificates issued by the country of origin are attached and attest that the country, zone, or export premises from which the fish originate is free of spring viraemia of carp (SVCV) and *Aeromonas salmonicida* (other than goldfish ulcer disease strains.)]

2. The goldfish are exported from a country, zone or export premises determined to be free from spring viraemia of carp virus (SVCV), and *Aeromonas salmonicida* (other than goldfish ulcer disease strains) based on a program of monitoring and surveillance acceptable to the Competent Authority, and consistent with criteria for health certification state stated in the DAFF import conditions as "Criteria on which additional veterinary certification for goldfish is based".

3. All goldfish in the consignment have been treated with an effective parasiticide (e.g. trichlorfon, formaldehyde, sodium chloride) during the seven (7) days prior to export to Australia to eliminate infestation by the gill flukes *Dactylogyrus vastator* and *D. extensus*.

[The active ingredients and concentration must be recorded on the veterinary certification.]

**Criteria on which additional veterinary certification for goldfish is based**

1. Spring viraemia of carp virus (SVCV): This certification must be based on negative test results on 3 batches of testing within the 6 month period immediately prior to export. The fish population from which the goldfish originate must be tested for SVCV using virus isolation and identification methods (virus neutralisation VN, immunofluorescence or enzyme-linked immunosorbent essay ELIZA), as described in the OIE 'Diagnostic Manual for Aquatic Animal Diseases'. Certification must be based on a minimum testing of 3 batches within the 6 month period immediately prior to export. The number of fish sampled must ensure a 95% confidence level of disease agent/pest detection in a source fish population with an agent prevalence of 2%. The population size is defined as the number of fish of the same species that share a common water supply and originate from the same broodstock.

[A statistics table is provided as part of the DAFF import permit.]

2. *Aeromonas salmonicida*: This certification must be based on negative test results on 3 batches of testing within the 6 month period immediately prior to export. The fish in the population from which the goldfish originate must be tested for *A. salmonicida* using standard bacteriological methods. Tissues tested should include skin lesions (if present), kidney, liver and spleen. Testing must be based on internationally accepted methods of isolation, culture and identification. Certification must be based on a minimum testing of 3 batches within the 6 month period immediately prior to export. The number of fish sampled must ensure a 95% confidence level of disease agent/pest detection in a source fish population with an agent prevalence of 2%. The population size is defined as the number of fish of the same species that share a common water supply and originate from the same broodstock.

[A statistics table is provided as part of the DAFF import permit.]

3. Certification of freedom from specified disease agents or pests (that is, SVCV, A. *salmonicida, Dactylogyrus vastator and D. extensus*) apply to the country, zone or export premises from which fish are exported to Australia. If the fish originated from a country other than the exporting country within 6 months of export, certification attesting to the health of the source fish population must accompany all shipments.

4. Countries, zones or premises declared free or in the process of establishing freedom from specified disease agents or pests, must take adequate precautions to prevent introduction of the disease agent or pest into the country, zone or premises, including via infected fish or water.

5. After two years of surveillance with laboratory tests and in the absence of suspect clinical signs or suspect positive test results, twice yearly testing must continue but the sample size at each inspection may be reduced to 30 fish of the species being exported (including broodfish when available), provided that all introductions of new fish to the population are sourced from fish populations that meet equivalent criteria or superior health standard.

**Standards for handling and packaging of live marine and freshwater ornamental fish for export to Australia**

1. All fish in the consignment must be packaged in accordance with International Air Transport Association (IATA) Live Animal Regulations.

2. All fish in the consignment must be packaged in leak-proof bags with each bag containing only one species. The bag must be colourless and sufficiently transparent to enable proper inspection and identification of the fish and must not contain any extraneous matter, unapproved plant material, pests or unauthorised species of fish. The use of outer bags of opaque materials or half-black bags to provide a dark shipping environment is acceptable, provided the contents of the bag can be properly inspected to the satisfaction of DAFF.

3. The inclusion of inert material such as zeolite, activated carbon, shredded plastic or dried terrestrial plants is permitted provided the contents of the bag can be properly inspected to the satisfaction of DAFF and the material is disinfected or destroyed as directed by DAFF.

4. The bags must be placed within polystyrene boxes or cartons fitted with a plastic lining. Each box or carton must be clearly identified as a part of a consignment and be individually identified.

5. The consignment must be accompanied by documents that include the identification number of each box or carton, and the scientific name and number of the contained fish. It is recommended that the common names of the fish also be included on the papers.

6. The fish in each bag must be stocked at a density that will facilitate inspection and hence must not be overcrowded. When packed for export, fish must be placed in clean water. The use of a pH indicator in the water is permissible, provided it does not interfere with inspection.

**Post arrival quarantine requirements for the importation of freshwater ornamental (aquarium) fish**

1. All shipments of ornamental finfish will be inspected by DAFF on arrival to ensure that they:

- are healthy
- the veterinary certification and invoice is in order
- are an approved species
- do not contain prohibited material or material of quarantine concern.

Fish not meeting these criteria and prohibited material will be seized or exported or destroyed at the importers expense.

2. All freshwater ornamental finfish will be ordered into quarantine at a DAFF approved freshwater ornamental fish quarantine premise QAP on arrival, for the following minimum periods:

- Goldfish: 21 days
- Gouramis and cichlids: 14 days
- Other freshwater ornamental finfish: 7 days

3. Where fish have been inspected by DAFF on arrival and found to satisfy all import conditions a movement direction will be issued by DAFF requiring the entire consignment of fish to be moved directly to the QAP named in the movement direction. Any significant event occurring during transport of the fish to the QAP e.g. accidents, loss of fish, loss of water must be reported to DAFF within 2 hours of the event.

4. Quarantine detention will be performed in accordance with Quarantine Approved Premise (QAP) criteria 7.1. under Section 46A of the Quarantine Act 1908.

[Standards for a QAP for the holding of ornamental fish can be obtained from regional DAFF offices.]

5. Based on fish species, country of origin, historical factors or any other relevant information, DAFF may test samples of imported fish during quarantine to determine their health status. The cost of such testing will be borne by the importer.

6. In the event of any imported fish showing clinical signs or producing a positive result to any tests indicating the presence of an infectious disease agent or pest, DAFF may cause any or all of the fish in the premises to be either detained in quarantine for further observation, testing and treatment, or be destroyed. Costs of any such action will be borne by the importer. If any fish are destroyed during any period of quarantine, compensation will not be paid by the Government.

7. Parties seeking to use alternative risk reduction measures to those listed in these conditions - for example, an extended period of quarantine detention or a specified testing regimen must obtain prior approval of alternative measures from DAFF. A submission including supporting scientific data that clearly explain the degree to which alternative measures would reduce risk, should be provided to DAFF for consideration.

8. At any time at the discretion of DAFF a permit/approval may be revoked or modified if these conditions are not met or if there is a change in quarantine risk.

9. At the end of the quarantine period, the fish will be inspected by DAFF and must be found free from clinical signs of pest and disease before they will be released from quarantine.

Note: A copy of the Import Risk Analysis (IRA) on which these import conditions are based is available from the Australia section of the DAFF website at Finalised Import Risk Analysis: Ornamental Finfish.

**List of permitted Live Freshwater fish suitable for Import**

All fish listings with size limits are to be measured using Standard Length (SL), which is the measurement from the snout to the end of the caudal peduncle

| **Taxon** | **Common name** |
|---|---|
| *Abramites hypselonotus* | Marbled headstander |
| *Acanthophthalmus* spp. | Kuhlii loach |
| *Aequidens pulcher* | Blue acara |
| *Alestopetersius caudalis* | Yellowtail Congo tetra |
| *Anostomus* spp. | Headstander |
| *Aphyocharax* spp. | Bloodfin tetras |
| *Aphyosemion* spp. | Killie fish |
| *Apistogramma* spp. | Dwarf cichlid |
| *Aplocheilus* spp. | Panchax |
| *Apteronotus albifrons* | Black ghost knifefish |
| *Apteronotus leptorhynchus* | Long nose brown ghost knifefish |
| *Arnoldichthys spilopterus* | Niger tetra, Arnold's characin, Red-eye characin |
| *Astronotus ocellatus* | Oscar |
| Only albino form of *Astyanax jordani* | Blind cave fish |
| *Aulonocara nyassae* of minimum length 5cm SL | African peacock cichlid, Emperor cichlid |
| *Aulonocara* spp. | African cichlids |
| Only male *Bagrichthys hypselopterus* | Black lancer catfish |
| *Balantiocheilos melanopterus* | Silver sharkminnow, Tricolor sharkminnow |

| | |
|---|---|
| *Bedotia geayi* | Madagascar rainbow, Red-tailed silverside |
| *Benthochromis tricot* | Benthochromis tricoti |
| *Betta* spp. | Fighting fish |
| *Boehlkea fredcochui* | Chochu's blue tetra |
| *Boraras maculates* | Dwarf spotted rasbora |
| *Botia lohachata* of minimum length 1.5cm SL | Reticulate loach, Yo-yo loach |
| *Brachygobius* spp. | Bumble bee fish |
| *Brochis* spp. | Blue catfish |
| *Brycinus longipinnis* | African tetra, Longfin tetra |
| *Campylomormyrus cassaicus* | Double-nose elephant nose |
| *Campylomormyrus rhynchophorus* | Double-nose elephant nose |
| *Carassius auratus auratus* | Goldfish |
| *Carnegiella* spp. | Hatchet fish |
| Only bridles morph *Chalinochromis brichardi* of minimum length 5cm SL | Lake Tanganyika cichlid |
| *Chalinochromis* spp. | Lake Tanganyika cichlids |
| *Chanda* spp. | Perchlets |
| *Chilodus punctatus* | Spotted headstander |
| *Chilotilapia rhoadesii* of minimum length 5cm SL | Rhoadesii cichlid, Bream |
| *Chromobotia macracanthus* | Clown loach |
| *Cleithracara maronii* | Keyhole cichlid |
| *Colisa chuna* | Honey dwarf gourami |
| *Colisa fasciata* | Giant dwarf gourami, Banded gourami |
| *Colisa labiosa* | Thick-lipped gourami |
| *Colisa lalia* | Dwarf gourami |
| *Copeina guttata* | Red spotted copeina |
| *Copella arnoldi* | Splash tetra, Characin, Jumping tetra |
| *Corydoras* spp. | Armoured catfish |
| Only males of *Corynopoma riisei* | Swordtail characin |
| *Crossocheilus siamensis* | Siamese flying fox, Siamese algae eater |
| *Cyathopharynx furcifer* | Featherfin cichlid, Thread fin furcifer |
| *Cyphotilapia frontosa* of minimum length 12cm SL | Frontosa, Humphead cichlid |
| *Cyprichromis leptosoma* | Yellowtail cyprichromis |
| *Cyrtocara moorii* | Lake Malawi cichlid |
| *Danio albolineatus* | Pearl danio |
| *Danio kerri* | Kerr's danio, Blue danio |
| *Danio nigrofasciatus* | Spotted danio, Dwarf danio |
| *Danio rerio* | Zebra danio, Leopard danio |
| *Dasyloricaria filamentosa* | Whiptail catfish |
| *Dekeyseria pulchra* of minimum length 3cm SL | Clown peckoltia, Butterfly pleco |
| *Dermogenys pusilla* | Half beak, Wrestling halfbeak |
| *Devario devario* | Bengal danio, Sind danio |
| *Devario malabaricus* | Malabar danio, Giant danio |
| *Dianema urostriatum* | Stripe tailed catfish |
| *Dicrossus filamentosus* | Checkerboard lyretail, Checkerboard cichlid, Chessboard cichlid |
| *Dicrossus maculatus* of minimum length 5cm SL | Checkerboard cichlid |
| *Epalzeorhynchos bicolor* | Redtail shark, Redtail sharkminnow |
| *Epalzeorhynchos frenatum* | Rainbow shark, Rainbow sharkminnow |
| *Epalzeorhynchos kalopterus* | Flying fox |
| *Epalzeorhynchos munense* | Redfin shark |
| *Epiplatys* spp. | Killie fish |
| *Eretmodus cyanostictus* | Dwarf goby cichlid, Tanganyika clown cichlid |
| *Esomus malayensis* | Flying barb, Malayan flying barb |
| *Farlowella acus* | Twig catfish, Whiptail catfish |
| *Gasteropelecus* spp. | Hatchet fish |
| *Glossolepis incisus* of minimum length 4cm SL | Red rainbow fish |
| *Gnathochromis permaxillaris* | African cichlid |

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|---|---|
| *Gnathonemus petersii* | Elephant nose |
| *Gymnocorymbus ternetzi* | Black widow tetra |
| *Gyrinocheilus aymonieri* | Sucking Asian catfish |
| *Hasemania nana* | Silver tip tetra |
| *Helostoma temminkii* | Green kissing gourami, Pink kissing gourami |
| *Hemigrammus* spp. | Tetras |
| *Hemiodus sterni* | Striped hemiodus |
| *Homaloptera orthogoniata* | Indonesian lizard fish |
| *Hyphessobrycon* spp. | Tetras |
| *Hypsophrys nicaraguense* of minimum length 5cm SL | Nicaraguan cichlid, Moga |
| *Inpaichthys kerri* | Blue emperor tetra, Royal tetra |
| *Iodotropheus sprengerae* | African cichlid, Rusty cichlid |
| *Julidochromis spp.* | Dwarf cichlid |
| *Kryptopterus bicirrhis* | Glass catfish |
| *Kryptopterus macrocephalus* | Poormans glass catfish, Striped glass catfish |
| *Labeo chrysophekadion* | Black shark |
| *Labeo cyclorhynchus* | Variegated shark, Harlequin shark |
| *Laetacara curviceps* | Curviceps, Flag acara |
| *Laetacara dorsigera* | Redbreast acara, Smiling acara |
| *Lamprologus ocellatus* of minimum length 5cm SL | Gold african cichlid, Gold ocellatus |
| *Laubuca laubuca* | Indian hatchet fish |
| *Lepidarchus adonis* | Flagtail tetra, Adonis tetra, Jellybean tetra |
| *Leporinus arcus* | Lipstick leporinus |
| *Leporinus fasciatus* | Banded leporinus |
| *Leporinus maculatus* | Spotted leporinus |
| *Leporinus multifasciatus* | Multi-banded leporinus |
| *Macrognathus aculeatus* | Spiny eel, Lesser spiny eel |
| Only male *Macropodus opercularis* of minimum length 6cm SL | Paradise fish |
| *Marcusenius macrolepidotus macrolepidotus* | Elephant nose, Bulldog |
| *Marosatherina ladigesi* | Celebes rainbow |
| *Megalamphodus* spp. | Tetras |
| *Melanochromis auratus* | Auratus, Golden mbuna |
| *Melanochromis simulans* | Auratus |
| Only non-Albino form of *Mesonauta festivus* | Festivum, Flag cichlid |
| *Metynnis* spp. of minimum length 4cm SL | Silver dollars |
| *Mikrogeophagus altispinosus* | Bolivian butterfly cichlid |
| *Mikrogeophagus ramirezi* | Ram cichlid |
| *Mimagoniates microlepis* | Croaking tetra, Blue tetra |
| *Moenkhausia* spp. | Tetras |
| *Monodactylus argenteus* | Angel mono, Malayan mono, Batfish, Silver moony |
| *Monodactylus sebae* | African mono, African moony |
| Only male *Myloplus rubripinnis* of minimum length 8cm SL | Red hook myleus |
| *Nannacara anomala* | Golden dwarf acara, Goldeneye cichlid |
| *Nannacara aureocephalus* | Golden head cichlid |
| *Nannacara taenia* | Dwarf lattice cichlid |
| *Nannostomus* spp. | Pencil fish |
| *Nematobrycon* spp. | Emperor tetra |
| *Neolamprologus brichardi* | Princess of Burundi |
| *Neolamprologus cylindricus* | Tanganyikan cichlid |
| Only yellow morph of *Neolamprologus leleupi* of minimum length 5cm SL | Lemon cichlid |
| *Neolamprologus meeli* of minimum length 5cm SL | African cichlid |
| *Neolamprologus mustax* of minimum length 5cm SL | Mustax, Mask lamprolagus |
| *Ophthalmotilapia* spp. | Blacknosed threadfin cichlid |
| *Oryzias latipes* | Golden medaka, Japanese rice fish |
| *Osteochilus hasseltii* | Bony lipped barb, Silver sharkminnow |

| | |
|---|---|
| *Osteochilus vittatus* | Bony lipped barb |
| *Otocinclus arnoldi* | Sucker catfish |
| *Pantodon buchholzi* | Butterfly fish |
| *Paracheirodon axelrodi* | Cardinal tetra |
| *Paracheirodon innesi* | Neon tetra |
| *Parachela oxygastroides* | Glass barb |
| *Paracyprichromis nigripinnis* | Blue neon cyprichromis |
| Only male *Parosphromenus deissneri* of minimum length 4cm SL | Licoricegourami |
| *Pelvicachromis pulcher* | Rainbow kribensis |
| *Pelvicachromis subocellatus* | Eye-spot kribensis, Eye-spot cichlid |
| *Pelvicachromis taeniatus* | Striped kribensis, Nigerian cichlid |
| *Petitella georgiae* | False rummy nose |
| *Petrochromis trewavasae trewavasae* of minimum length 5cm SL | Texas cichlid, White spotted peerchromis, Threadfin cichlid |
| *Phenacogrammus interruptus* | Congo tetra |
| *Pimelodus ornatus* | Ornate pimelodus catfish |
| *Pimelodus pictus* | Pictus catfish |
| *Poecilia latipinna* | Sailfin mollie |
| *Poecilia reticulata* | Guppy |
| *Poecilia sphenops* | Black mollie |
| *Poecilia velifera* | Yucatan sailfin mollie |
| *Only male Poecilocharax weitzmani* | Shining tetra |
| *Prionobrama filigera* | Glass bloodfin |
| *Pristella maxillaris* | Pristella, X-ray tetra |
| *Pseudogastromyzon myersi* | Dwarf stone sucker, Sucker-belly loach |
| Only male *Pseudomystus siamensis* of minimum length 8cm SL | Siamese catfish, Bumble bee catfish |
| *Pterophyllum* spp. | Angel fish |
| *Puntius arulius* | Longfin barb, Arulius barb |
| *Puntius asoka* | Asoka barb |
| *Puntius bimaculatus* | Two spot barb, Redside barb |
| *Puntius conchonius* | Rosy barb |
| *Puntius cumingii* | Cummings barb, Two spot barb |
| *Puntius everetti* | Clown barb |
| *Puntius fasciatus* | Striped barb |
| *Puntius filamentosus* | Black spot barb |
| *Puntius hexazona* | Tiger barb |
| *Puntius lateristriga* | Spanner barb |
| *Puntius lineatus* | Striped barb |
| *Puntius nigrofasciatus* | Ruby barb |
| *Puntius oligolepis* | Checkered barb |
| *Puntius partipentazona* | Tiger barb |
| *Puntius pentazona* | Five-banded barb |
| *Puntius semifasciolatus* of minimum length 3cm SL | Golden barb, Chinese barb |
| *Puntius tetrazona* | Tiger barb, Sumatra barb |
| *Puntius ticto* | Ticto barb |
| *Puntius titteya* | Cherry barb |
| *Puntius vittatus* | Kooli barb, Greenstripe barb |
| *Rasbora argyrotaenia* | Silver rasbora |
| *Rasbora borapetensis* | Red tail rasbora, Blackline rasbora |
| *Rasbora caudimaculata* | Red tail rasbora, Greater scissortail |
| *Rasbora dorsiocellata* | Emerald eye rasbora, Eyespot rasbora |
| *Rasbora dusonensis* | Yellow tail rasbora, Rosefin rasbora |
| *Rasbora einthovenii* | Blue line rasbora, Brilliant rasbora |
| *Rasbora elegans* | Two spot rasbora |
| *Rasbora kalochroma* | Clown rasbora |
| *Rasbora leptosome* | Copper striped rasbora |

| | |
|---|---|
| *Rasbora pauciperforata* | Red line rasbora |
| *Rasbora sarawakensis* | Sarawak rasbora |
| *Rasbora steineri* | Gold line rasbora, Chinese rasbora |
| *Rasbora trilineata* | Black scissortail, Three-lined rasbora |
| *Rasbora vaterifloris* | Flame rasbora, Pearly rasbora |
| *Rhodeus amarus* | European bitterling |
| *Rhodeus sericeus* | Amur bitterling |
| *Sawbwa resplendens* of minimum length 1.5cm SL | Asian rummynose, Sawbwa barb |
| *Semaprochilodus insignis* | Flagtail prochilodus |
| *Semaprochilodus taeniurus* | Silver prochilodus |
| *Spathodus erythrodon* | Blue spotted goby cichlid |
| *Sphaerichthys osphromenoides* | Chocolate gourami |
| Only female *Sturisoma panamense* of minimum length 8cm SL | Armoured catfish |
| *Symphysodon* spp. | Discus |
| Only male *Synodontis decora* of minimum length 10cm SL | Clown squeaker catfish |
| *Synodontis multipunctata* | Cuckoo catfish, African catfish |
| *Synodontis nigriventris* | Upsidedown catfish, Blotched upsidedown catfish |
| *Tanganicodus irsacae* | Goby cichlid, Spotfin goby cichlid |
| *Tanichthys albonubes* | White cloud, Mountain minnow |
| *Tateurndina ocellicauda* | Peacock gudgeon |
| *Thayeria* spp. | Hockeystick tetra |
| *Thoracocharax* spp. | Hatchet fish |
| *Toxotes jaculatrix* | Banded archer fish |
| Only male *Trachelyopterus fisheri* of minimum length 7cm SL | Woodcat, Driftwood catfish |
| *Trichogaster leeri* | Pearl gourami |
| *Trichogaster microlepis* | Moonbeam gourami |
| *Trichogaster trichopterus* | Golden gourami, Three spot gourami |
| *Trichopsis pumila* | Pygmy gourami |
| *Trichopsis vittata* | Croaking gourami |
| *Trigonostigma hengeli* | Harlequin rasbora, Glowlight rasbora |
| *Trigonostigma heteromorpha* | Harlequin rasbora |
| *Trinectes maculates* | Freshwater flounder, Hogchocker |
| *Triportheus* spp. | False hatchet |
| *Tropheus* spp. of minimum length 3cm SL | African cichlids |
| *Xiphophorus hellerii* | Swordtail |
| *Xiphophorus maculates* | Platy |
| *Xiphophorus variatus* | Variegated platy |
| *Yasuhikotakia sidthimunki* of minimum length 1.5cm SL | Dwarf botia, Skunk loach |

Only live ornamental fish included in the DAFF permitted species list are eligible for importation into Australia from approved countries.

Importers are advised to check that this list is current at the time of importation. Only fish appearing on this list on the day of import will be eligible for entry into Australia.

A current list of DAFF permitted live ornamental fish can be found at the ICON database.

Condition C9312

**List of Competent Authorities**

**Bahrain - Marine fish only**
Ministry of Commerce
Kingdom of Bahrain

**Belgium**
Federal Agency for the Security of the Food Chain
Belgium

**China**
Animal Quarantine Division
Department of Supervision for Animal and Plant Quarantine
General Administration of Quality Supervision, Inspection and Quarantine
People's Republic of China

**Federated States of Micronesia**
Division of Agriculture
Department of Resources and Development
Federated States of Micronesia

**Fiji**
Ministry of Agriculture, Fisheries and Forests
Fiji

**French Polynesia**
Ministry of Agriculture
French Polynesia

**Germany**
Veterinary issues in the export sector
Federal Ministry for Food, Agriculture and Consumer Protection
Germany

**Hong Kong**
Inspection and Quarantine Branch
Agriculture, Fisheries and Conservation Department
Hong Kong

**Indonesia**
Centre for Fish Quarantine
Ministry of Agriculture
Jakarta
Indonesia

**Indonesia – Bali**
Fish Quarantine Office, Denpasar
Ministry of Agriculture
Bali
Indonesia

**Indonesia- Jakarta**
Agricultural Quarantine Office, Soekarno-Hatta
Ministry of Agriculture
Jakarta
Indonesia

**Kenya**
Ministry of Agriculture, Livestock & Marketing
Kenya

**Malaysia**
Fisheries Biosecurity Division
Department of Fisheries
Malaysia

**New Caledonia**
Department Import/Export
Direction de l'Agricultureet de la Foret

New Caledonia

**New Zealand**
General Standards
Ministry of Agriculture and Forestry
NEW ZEALAND

**Phillipines**
Fish Health and Quality Management Assurance Section
Bureau of Fisheries and Aquatic Resources
Department of Agriculture
Republic of the Philippines

**Saudi Arabia**
Animal Quarantine
Ministry of Agriculture and Water
Kingdom of Saudi Arabia

**Senegal**
Ministry of Agriculture & Water Resources
Senegal

**Seychelles**
Veterinary Services Section
Animal Health & Development Division
Ministry of Agriculture & Marine Resources
Seychelles

**Singapore**
Agri-Food and Veterinary Authority
Singapore

**Solomon Islands**
Quarantine
Ministry of Agriculture and Fisheries
Solomon Islands

**South Africa**
Import Export Policy Unit
Animal Health Directorate
Department of Agriculture, Forestry and Fisheries
Republic of South Africa

**Sri Lanka -** (excluding *Carassius auratus auratus* - Goldfish)
Department of Animal Production and Health
Sri Lanka

**Tawain – Marine fish only**
Bureau of Animal and Plant Health Inspection and Quarantine
Taiwan

**Tanzania – Marine fish only**
Ministry of Agriculture and Cooperatives
Government of Tanzania
Tanzania

**Thailand**
Department of Fisheries
Thailand

**USA (includes Hawaii)**
Imports and Exports of Live Animals
Animal and Plant Health Inspection Service

United States Department of Agriculture
USA

**Vanuatu- Marine fish only**
Vanuatu Quarantine and Inspection Service
Vanuatu

Entry Management EM0186

| Commodity Docs | Direction | AIMS Comments | Notes / QAP |
|---|---|---|---|
| **Mandatory:**<br><br>1) A valid **Import Permit** - see permit conditions for specific requirements.<br><br>2) **Health Certificate**- see permit conditions for specific requirements<br><br>**NOTE:** Import permit requirements outweigh ICON conditions and work instructions unless otherwise stated within the permit. | **All Docs OK** | | |
| | **All Cargo Types - valid permit, health certificate and packing list** – ***Inspection occurring at CTO*** | | |
| | 1) Documentation - Present all Documentation | | |
| | 2) Inspection Direction – Inspect Live Fish at CTO | **Standard Comments for Inspection Direction**<br><br>Fish may not move from the CTO until inspected by a Quarantine Officer.<br><br>Present original health certificate(s) and valid import permit (or copy) or means to allow the identification of the import permit. | 1.1, 1.2 |
| | 2) Relevant Isolation Direction(s)<br><br>a) 7 Days Q'tine of Live Fish<br><br>b) 14 Days Q'tine of Live Fish<br><br>c) 21 Days Q'tine of Live Fish | **Standard Comments for Isolation Direction**<br><br>Live Fish to be moved directly to QAP for isolation in accordance with import permit conditions.<br><br>OIQ # | A written order into quarantine or AIMS order into quarantine to the importer is required to accompany Isolation Directions |
| | 3) Inspection Direction – Live Fish Inspect @ QAP | **Standard Comments for Inspection Direction**<br><br>Final inspection of Live Fish after isolation period of <number> days.<br><br>All fish are to remain isolated until inspected and released by DAFF. | 7.1 |
| | **All Docs OK** | | |
| | **All Cargo Types - valid permit, health certificate and packing list** – ***Inspection occurring at DAFF Office*** | | |

| | | | |
|---|---|---|---|
| | 1) Documentation - Present all Documentation | Present all original documents to nearest DAFF Regional Office | |
| | 2) Inspection Direction -Seal/ChkCtns Live Fish @ CTO | **Standard Comments for Inspection Direction**<br><br><numberctns> Live Fish ex <country of origin>. Must be <sealed with Quarantine Seals/checked by Quarantine Officer> prior to movement from bond.<br><br>Once advised by Q Officer – move fish directly to nearest DAFF Regional Office. | 1.1, 1.2 |
| | 3) Inspection Direction –<br><br>Inspect Live Fish @DAFF Office | **Standard Comments for Inspection Direction**<br><br>Fish will be subject to unpack and inspection.<br><br>Present valid import permit (or copy) or a means of identification of the import permit and original health certificate(s) at inspection. | DAFF Office<br><br>A written order into quarantine or AIMS order into quarantine to the importer is required to accompany Isolation Directions |
| | 4) Relevant Isolation Direction(s)<br><br>a) 7 Days Q'tine of Live Fish<br><br>b) 14 Days Q'tine of Live Fish<br><br>c) 21 Days Q'tine of Live Fish | **Standard Comments for Isolation Direction**<br><br>Live Fish to be moved directly to QAP for isolation in accordance with import permit conditions.<br><br>OIQ # | 7.1 |
| | 5) Inspection Direction – Live Fish Inspect @ QAP | **Standard Comments for Inspection Direction**<br><br>Final inspection of Live Fish after isolation period of <number> days.<br><br>All fish are to remain isolated until inspected and released by DAFF. | |
| | **All Docs NOT OK** | | |
| | **All Cargo Types, nil valid health certificate, nil valid import permit, nil valid packing list** | | |
| | 1) Documentation - Present all Documentation | **Standard Comments for Documentation Direction**<br><br>Present all documents including a valid <import permit/health | DAFF supervisor will be notified.<br><br>The consignment must |

| | | | |
|---|---|---|---|
| | | certificate/packing list> for <commodity> as per ICON.<br><br>Alternatively the goods may be exported or destroyed at importer's expense. | be exported or destroyed |

Import Permit Fee IPF0005

**Import Permit Fees (where applicable) – Category 4**

This commodity is classified as a Category 4 assessment for the purposes of determining the Import Permit fee rate that applies. The fee rate is $240.00 (for any assessment period up to 3 hours) and $40.00 for each quarter hour, or part of a quarter hour, after the 3-hour period. Note that in addition to the assessment fee, an electronic lodgement fee of $85.00 or a manual lodgement fee of $150.00 also applies.

An assessable item means an item identified on an Import Permit application as consisting of goods of a class imported, or to be imported, from a particular country for a particular use.

Further information on DAFF fees and charges can be found on the DAFF website. Import Permit issuing fees are specified in the Quarantine Service Fees Determination 2005.

# APPENDIX 6: BIOSECURITY AUSTRALIA ADVICE NOTICES RELATING TO THIS PROJECT

## i. BAA 2008/29

Australian Government
Biosecurity Australia

11 September 2008

**BIOSECURITY AUSTRALIA ADVICE 2008/29**

**COMMENCEMENT OF AN IMPORT RISK ANALYSIS FOR ORNAMENTAL FINFISH WITH RESPECT TO GOURAMI IRIDOVIRUS**

This Biosecurity Australia Advice announces the formal commencement of an Import Risk Analysis (IRA) under the regulated IRA process to review Australia's freshwater ornamental finfish policy with respect to quarantine risks associated with gourami iridovirus (GIV).

This analysis will be undertaken as a standard IRA, requiring completion within 24 months from announcement.

Biosecurity Australia is presently preparing a draft IRA report which will be circulated to stakeholders shortly for comment. Stakeholders will have up to 60 days to submit written comments on the draft report and will have opportunities to discuss matters raised in any submissions directly with Biosecurity Australia.

Biosecurity Australia Policy Memorandum 2007/20 of 12 September 2007 advised stakeholders that changes to the IRA process had been implemented on 5 September 2007 when regulations made under the *Quarantine Act 1908* formally took effect. That advice also notified the transitional arrangements for Biosecurity Australia's import work program, including a number of risk analyses that commenced under the previous arrangements and were to transition to the new regulated IRA process.

A policy review on the importation of live freshwater ornamental finfish with respect to iridoviruses had previously been announced. Stakeholders were informed of this in Animal Biosecurity Policy Memorandum 2005/01 of 11 March 2005. This policy review will now be completed under the new regulated IRA process as a standard IRA.

Gouramis are a group of attractive ornamental fish comprising several species native to South East Asia and Africa. They are sought after aquarium species. Australia has imported a large number of gouramis for many decades. Ornamental finfish are imported under a range of quarantine conditions established by an IRA completed in 1999.

Iridoviruses can cause serious disease in fish. The 1999 IRA considered several species of gouramis and concluded, amongst other things, that specific risk management measures were required for these species due to biosecurity risk posed by iridoviruses, including GIV. Australia's quarantine measures include that gouramis are held in an export premises for a minimum 14 day period prior to export, health certification that they are sourced from populations with no known significant clinical disease in the last six months, and that the fish are held in post-arrival quarantine for a minimum of 14 days. Evidence, including research by the University of Sydney, indicates possible changes to the understanding of GIV and this prompted the commencement of the policy review in 2005.

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DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY

Following the announcement of a policy review on 11 March 2005, considerable work has been undertaken to progress this analysis. Biosecurity Australia therefore expects to release a draft IRA report shortly to enable all interested stakeholders to provide submissions and comment.

In announcing this IRA, Biosecurity Australia has considered advice from the Import Market Access Advisory Group, which was established as part of the revised IRA arrangements to advise Biosecurity Australia on priorities for import proposals.

The regulations require the Chief Executive of Biosecurity Australia to announce formally the commencement of IRAs to be undertaken under the regulated process. This announcement triggers the start of the regulated timeframe for an IRA.

Further information on the new regulated IRA process can be found on Biosecurity Australia's website www.biosecurityaustralia.gov.au.

Dr Colin J Grant
Chief Executive

Contact officer: Dr Robert Heard
Telephone: 02 6272 4836
Facsimile: 02 6272 3399
E-mail: Robert.Heard@biosecurity.gov.au

## ii. BAA 2009/06

Australian Government
Biosecurity Australia

24 March 2009

**BIOSECURITY AUSTRALIA ADVICE 2009/06**

**DRAFT IMPORT RISK ANALYSIS REPORT FOR ORNAMENTAL FINFISH WITH RESPECT TO IRIDOVIRUSES**

This Biosecurity Australia Advice (BAA) notifies stakeholders of the release of the *Draft import risk analysis report for freshwater ornamental finfish with respect to quarantine risks associated with gourami iridovirus and other related viruses*.

The draft import risk analysis (IRA) report assesses the risks associated with imports of ornamental finfish and proposes that additional quarantine measures are required to manage the quarantine risks in accordance with Australia's conservative approach to quarantine. The IRA recommends fish be sourced from populations demonstrated to be free of the iridoviruses of quarantine concern, or batch-tested on arrival to show they are free of iridoviruses of quarantine concern.

The draft IRA report is being issued for 60 days consultation. Written comments will need to be provided to Biosecurity Australia by 25 May 2009.

On 11 September 2008, Biosecurity Australia announced the formal commencement of a standard, regulated IRA to assess the current quarantine policy for the importation of live freshwater ornamental finfish with respect to gourami iridovirus and related viruses (BAA 2008/29). This BAA notifies stakeholders of the release of the *Draft import risk analysis report for freshwater ornamental finfish with respect to quarantine risks associated with iridoviruses and other related viruses*. Under the Quarantine Regulations 2000, stakeholders have 60 days, until 25 May 2009, to provide written comments on the draft IRA report.

Gouramis, cichlids (such as angelfish and oscars) and poeciliids (such as guppies and platys) are popular aquarium species. Ornamental finfish are imported under a range of quarantine conditions based on an IRA completed in 1999. Australia's quarantine measures include that the fish are held in an export premises for a minimum 14-day period prior to export, health certification that they are sourced from populations with no known significant disease in the last six months, and that the gouramis and cichlids are held in post-arrival quarantine for a minimum of 14 days and poeciliids for a minimum of seven days. Millions of ornamental fish are imported into Australia each year.

Evidence, including research by the University of Sydney, has indicated possible changes to the understanding of iridoviruses. This prompted the commencement of a policy review in 2005. The policy review is being completed as a regulated IRA.

The draft IRA report recommends additional tighter quarantine measures. These are:

- sourcing fish from populations demonstrated to be free of the iridoviruses of quarantine concern; or
- batch-testing fish on arrival to show they are free of iridoviruses of quarantine concern.

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The existing pre-export quarantine of period of 14 days and relevant official health certification would apply, together with a post arrival quarantine period of seven days.

The draft IRA report and information about the regulated IRA process are available from the Biosecurity Australia website, www.biosecurityaustralia.gov.au. Printed copies are available, if required. Comments on the draft IRA report need to be submitted by 25 May 2009 to:

Animal Biosecurity
Biosecurity Australia
GPO Box 858
CANBERRA ACT 2601
E-mail: animal@biosecurity.gov.au

Stakeholder comments will be carefully considered and a provisional final report will be prepared. The provisional final report will be open to appeal. Following the appeal process, the final report and recommendations will be provided to Australia's Director of Animal and Plant Quarantine to make a quarantine policy determination.

**Confidentiality**

Stakeholders are advised that, subject to the *Freedom of Information Act 1982* and the *Privacy Act 1988*, all submissions received in response to BAAs will be publicly available and may be listed or referred to in any papers or reports prepared on the subject matter.

The Commonwealth of Australia reserves the right to reveal the identity of a respondent unless a request for anonymity accompanies the submission. Where a request for anonymity does not accompany the submission the respondent will be taken to have consented to the disclosure of their identity for the purposes of Information Privacy Principle 11 of the Privacy Act.

The contents of the submission will only be treated as confidential if they are marked 'confidential' and can be classified as such in accordance with the Freedom of Information Act.

Dr Colin J Grant
Chief Executive

Contact officer: Dr Ramesh Perera
Telephone: +61 2 6272 4675
Facsimile: +61 2 6272 3399
E-mail: Ramesh.Perera@biosecurity.gov.au

## iii. BAA 2010/22

Australian Government
Biosecurity Australia

22 July 2010

**BIOSECURITY AUSTRALIA ADVICE 2010/22**
**PROVISIONAL FINAL IMPORT RISK ANALYSIS REPORT FOR FRESHWATER ORNAMENTAL FINFISH WITH RESPECT TO GOURAMI IRIDOVIRUS AND RELATED VIRUSES**

This Biosecurity Australia Advice notifies stakeholders of the release of the *Provisional final import risk analysis report for freshwater ornamental finfish with respect to the quarantine risks associated with gourami iridovirus and related viruses.*

The provisional final import risk analysis (IRA) report recommends that the importation of fish of the gourami, cichlid and poeciliid families for ornamental purposes be permitted if the fish are batch tested post-arrival in Australia to show they are free of megalocytiviruses; or are sourced from a country, zone or compartment that is recognised by Australia to be free of megalocytiviruses.

The provisional final report takes into account submissions and comments by stakeholders on a draft report released in March 2009. The report is open for appeal to the independent Import Risk Analysis Appeals Panel until 23 August 2010.

Biosecurity Australia Advice 2008/29 of 11 September 2008 announced the formal commencement of a standard IRA under the regulated process to consider the quarantine risks associated with the importation of freshwater ornamental finfish with respect to gourami iridovirus and related viruses. Comments from 18 stakeholders on the draft IRA report issued on 24 March 2009 were taken into account in preparing the provisional final report.

Changes have been made to the IRA report as a result of stakeholder comments and new scientific information that has come to light. Consequently, Biosecurity Australia has reassessed the risks associated with ranaviruses and megalocytiviruses separately. As a result, risk management measures for poeciliid ranavirus are no longer recommended. A recent report of megalocytivirus in paradise fish has led to the addition of this subfamily of the gourami family to the fish that are subject to the recommended risk management measures.

The provisional final IRA recommends that the importation of fish of the gourami, cichlid and poeciliid families for ornamental purposes be permitted only if

- fish are batch tested post-arrival in Australia 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).

This IRA is being completed under the regulated process according to the *Import Risk Analysis Handbook 2007 (update 2009).* The release of this provisional final IRA report marks the end of the regulated timeframe for the IRA. The provisional final IRA report and information about the regulated

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DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY

IRA process are available from www.biosecurityaustralia.gov.au. Printed copies are available on request.

Stakeholders may lodge an appeal in writing to the Import Risk Analysis Appeals Panel—a body independent of Biosecurity Australia. The panel has advised that any appeals must be received by 23 August 2010. The appeal must outline a claim or claims based on the ground that there was a significant deviation from the regulated IRA process that adversely affected the interests of a stakeholder. Each claim must be supported by a statement of reasons.

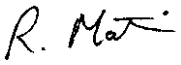
The panel has 45 days to consider any appeals and report its findings to Australia's Director of Animal and Plant Quarantine and appellants. If there is no appeal, or once any appeals are resolved, the process is complete and policy recommendations will be submitted to the Director of Animal and Plant Quarantine for consideration of the determination.

Written appeals (by email or post) must be addressed to the Import Risk Analysis Appeals Panel secretariat, as follows:

Secretariat
Import Risk Analysis Appeals Panel
Department of Agriculture, Fisheries and Forestry
GPO Box 858
CANBERRA ACT 2601

Email: IRAAP@daff.gov.au

Further details on the appeal process can be found in the *Import Risk Analysis Handbook 2007 (update 2009)* at www.daff.gov.au/irahandbook and from the IRAAP secretariat at http://www.daff.gov.au/iraap.



for Dr Colin J Grant
Chief Executive

Contact: Dr Ramesh Perera
Tel: +61 2 6272 3933
Fax: +61 2 6272 3399

## iv. BAA 2011/16

Australian Government
Department of Agriculture, Fisheries and Forestry

1 September 2011

**BIOSECURITY AUSTRALIA ADVICE 2011/16**

**CONDITIONS FOR THE IMPORTATION OF LIVE FRESHWATER ORNAMENTAL FINFISH INTO AUSTRALIA: AMENDED HEALTH CERTIFICATION REQUIREMENTS FOR GOLDFISH**

This Biosecurity Australia Advice notifies stakeholders of amended health certification requirements for the importation of goldfish (*Carassius auratus*) for ornamental purposes. Recent research findings have confirmed that Australia's aquatic animal health status with regard to goldfish haematopoietic necrosis virus (GFHNV) has changed and is now present in Australia. The requirement that exporting countries declare that goldfish intended for Australia are free from the disease is to be removed from relevant importation conditions.

Recent research findings have indicated that goldfish haematopoietic necrosis virus (GFHNV) is present in domestic goldfish populations in Australia. It will no longer be necessary for goldfish exported to Australia to be certified free of the disease.

Import conditions for ornamental finfish, including exporting country health certification attesting to GFHNV freedom in goldfish consignments, were established following completion of the 1999 *Import Risk Analysis on Live Ornamental Finfish*, which found that GFHNV was exotic to Australia. Health certification attesting to freedom from the disease was therefore warranted. Since 1999, reports of domestic goldfish exhibiting clinical and histopathological signs indicative of GFHNV infection have been published. However, these findings were not associated with epizootics. The recent development of a molecular (polymerase chain reaction) method for GFHNV identification has enabled subsequent testing of domestic goldfish populations in farms and at wholesale and retail outlets in Australia. This work has established that GFHNV is now present in Australia. Following consideration of these findings, Animal Biosecurity Branch has reviewed importation conditions for goldfish and concluded that health certification requirements in relation to GFHNV should be discontinued.

The revised health certification requirements for goldfish are provided at Attachment 1. A revised model health certificate is provided at Attachment 2.

Please pass this notice to other interested parties. If those parties wish to be included in future communications on this matter they should contact Animal Biosecurity Branch at animal@daff.gov.au . Information on risk assessments and policy reviews being conducted by Animal Biosecurity Branch are available on the Department's website.

ANDREW CUPIT
A/g General Manager
Animal Biosecurity Branch

GPO Box 858 (18 Marcus Clarke Street) Canberra City ACT 2601 Location: 7 London Circuit Tel +61 2 6272 3933 www.daff.gov.au/ba

## v. BAA 2012/01

Australian Government
Department of Agriculture, Fisheries and Forestry

12 January 2012

**BIOSECURITY ADVICE 2012/01**

**IMPORT RISK ANALYSIS FOR FRESHWATER ORNAMENTAL FINFISH WITH RESPECT TO GOURAMI IRIDOVIRUS AND RELATED VIRUSES**

This Biosecurity Advice notifies stakeholders of the status of the import risk analysis (IRA) for freshwater ornamental finfish with respect to the quarantine risks associated with gourami iridovirus and related viruses. The Director of Animal and Plant Quarantine has considered the IRA report and will await the completion of a University of Sydney survey of Australian fish for megalocytivirus before making a determination on the IRA's recommendations. The survey will provide additional information about the disease status of Australian fish with respect to megalocytivirus.

The provisional final IRA report was issued on 22 July 2010 for a 30-day appeal period. In October 2010 the Import Risk Analysis Appeals Panel advised the three appellants and the Director of Animal and Plant Quarantine that it had disallowed six claims and found the other to be outside the ground for appeal.

Following completion of the appeal process, the Director of Animal and Plant Quarantine has considered the final IRA report. The Director notes that the estimation of risk in the IRA report is based on the assumption that farmed and wild Australian fish are free of megalocytivirus, and that this assumption is based on limited data. He is also aware of a Fisheries Research and Development Corporation funded survey of Australian fish for megalocytivirus currently being undertaken by the University of Sydney. The survey is due for completion in March 2013. Given that this survey will provide additional relevant information, the Director of Animal and Plant Quarantine has decided to await the survey's outcomes before making a policy determination.

The current import conditions for freshwater ornamental fish with respect to iridovirus will remain in place until further notice.

Andrew Cupit
A/g Assistant Secretary
Animal Biosecurity

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## vi. BAA 2012/23

Australian Government
Department of Agriculture, Fisheries and Forestry
Biosecurity

15 November 2012

**BIOSECURITY ADVICE 2012/23**

**TRIAL OF A PROPOSED NEW SYSTEM FOR MANAGING THE BIOSECURITY RISKS OF IMPORTED ORNAMENTAL FISH**

This Biosecurity Advice informs stakeholders of a trial of proposed changes to Australia's system for managing biosecurity risks associated with imported ornamental fish. The proposed changes aim to better manage the biosecurity risks by shifting the emphasis of risk management off-shore. The changes would negate the need for the current system of post-arrival quarantine detention of fish. The proposed changes include an on-arrival fish health surveillance program that would continuously monitor the effectiveness of overseas authorities in ensuring the health of ornamental fish exported to Australia. The department is planning to trial the surveillance system to test its operational feasibility. Ornamental fish import permit holders willing to participate in the trial are asked to contact Animal Biosecurity by 17 December 2012.

The department is proposing changes to the way it manages the disease risks associated with imported ornamental fish, placing greater emphasis on managing the biosecurity risks off-shore at source. The proposed changes include the introduction of an on-arrival fish health surveillance program that would allow DAFF to monitor the performance of overseas authorities and export establishments and ensure that the health requirements of ornamental fish exported to Australia are met. These arrangements should also enable the department to be more responsive to emerging disease issues and to work closely with exporting countries to manage biosecurity risks effectively.

Under the proposed system;

- all shipments of freshwater and marine ornamental fish would continue to be inspected on arrival,
- bags of fish showing significant mortality or morbidity would be sampled during on-arrival inspection and the remaining fish destroyed or re-exported,
- some bags would be selected randomly during on-arrival inspection for sampling and the remaining fish in the consignment released,
- fish samples would be sent to participating laboratories for a general health assessment and testing for specific disease agents of quarantine concern, and
- the information obtained would be analysed and appropriate action taken to manage any biosecurity disease risk at the source.

Under the proposed system there would not be a need for Class 7.1 quarantine approved premises (QAP) for the post-arrival detention of imported ornamental fish. Should the new arrangements be introduced, the department would work with premises operators to manage this process, which could coincide with the annual licence renewal process.

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The department is planning to trial the on-arrival fish health surveillance program to test its operational feasibility. The first trial will involve only those bags of fish that would otherwise be destroyed due to non-compliance with Australian import requirements. A subsequent more comprehensive trial is planned for which DAFF will need the cooperation of volunteer importers. Holders of ornamental fish import permits who are willing to participate in the trial are asked to contact Animal Biosecurity by 17 December 2012.

The contact is:

Ramesh Perera
Animal Biosecurity
Department of Agriculture, Fisheries and Forestry
GPO Box 858
CANBERRA ACT 2601
Telephone: +61 2 6272 4675
Facsimile: +61 2 6272 3399
Email: animal@daff.gov.au

All current import conditions and requirements will remain in place during the trial. While the trial is being conducted and assessed, DAFF has suspended work on the review and amendment process of the Class 7.1 QAP criteria.

Please pass this notice to other interested parties. If those parties wish to be included in future communications on this matter they should contact Animal Biosecurity.

Andrew Cupit
Assistant Secretary
Animal Biosecurity