Experimental transmission and virulence of a megalocytivirus (Family Iridoviridae) of dwarf gourami (Colisa lalia) from Asia in Murray cod (Maccullochella peeli peeli) in Australia

Jeffrey Go, Richard Whittington *

OIE Reference Laboratory for EHN Virus, Faculty of Veterinary Science, The University of Sydney, NSW 2006, Australia

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Abstract

In February 2003 there were 90% losses of Murray cod (Maccullochella peeli peeli) fingerlings in a Victorian aquaculture facility. The disease was caused by a megalocytivirus (Family Iridoviridae) closely related to the recognized species Infectious Spleen and Kidney Necrosis Virus (ISKNV) and strain dwarf gourami iridovirus (DGIV), neither of which had previously been reported from farmed or wild fish in Australia. Experimental transmission trials were undertaken to test the hypothesis that the outbreak could have arisen through introduction of a virus with ornamental gouramis imported from South East Asia. Intraperitoneal injection of Murray cod fingerlings using filtered tissue homogenates from dwarf gourami (Colisa lalia) positive for megalocytivirus DNA by polymerase chain reaction (PCR) resulted in >90% mortality. Mortality was also induced by cohabitating Murray cod fingerlings and dwarf gouramis; as the fish were physically separated, the virus spread between the two species via water. Histopathology revealed lesions identical to those reported in the Victorian outbreak, 125–130 nm icosahedral virions were observed in lesions and most exposed fish were PCR positive. DNA sequencing confirmed 99.9 to 100% homology of major capsid protein and ATPase nucleotide sequences between DGIV, ISKNV, the viral inoculum obtained from dwarf gourami and virus present in experimentally infected Murray cod. These findings confirm that Murray cod are highly susceptible to a megalocytivirus present in ornamental fish imported from South East Asia. The implications for aquaculture, conservation of native fish and quarantine policy are discussed.

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Keywords: Megalocytivirus; Iridovirus; DGIV; Ornamental fish; Disease transmission; Maccullochella peeli peeli; Gourami

1. Introduction

The Murray cod (Maccullochella peeli peeli), a threatened species, is found only in the Murray-Darling river system of south-eastern Australia. The species has high conservation value and iconic status. Recently an aquaculture industry based on the Murray cod has developed, and commercial quantities of this species are produced for local markets (Weston et al., 2001). In February 2003 a mass mortality event occurred in Murray cod on a farm in Victoria. It was due to a virus in the genus Megalocytivirus of the Family Iridoviridae (Go et al., 2006) which consists of icosahedral double stranded DNA viruses 120–350 nm in diameter (Chinchar et al., 2005). Megalocytiviruses produce...
protozoan-like basophilic inclusions in hypertrophied cells in many organs and are difficult to cultivate in vitro.

Megalocytiviruses cause significant diseases in farmed marine finfish in Asia. For example, Red Sea bream iridovirus (RSIV) caused mass mortality in 30 species in Japan (Inouye et al., 1992; Kawakami and Nakajima, 2002). Similar viruses have been identified from a range of grouper species (Epinephelus spp.) cultured in Taiwan and South-East Asia (Chou et al., 1998; Danayadol et al., 1997; Kasornchandra and Khongpradit, 1997; Qin et al., 2003), beakperch (Oplegnathus fasciatus) and rockfish (Sebastes schlegeli) cultured in Korea (Jung and Oh, 2000; Kim et al., 2002), and large yellow croaker (Lateolabrax crocea) (Chen et al., 2003), red drum (Sciaenops ocellatus) (Weng et al., 2002) and turbot (Shi et al., 2004) cultured in China. Megalocytiviruses have also been implicated in “sleepy grouper disease” in Epinephelus spp. (Sudthongkong et al., 2002a), and “sea bass iridoviral disease” in Lateolabrax sp. (Matsuoka et al., 1996). Infectious Spleen and Kidney Necrosis Virus (ISKNV) caused mass mortality in mandarin fish (Siniperca chuatsi), a freshwater food fish cultured in China (He et al., 2000).

Megalocytiviruses also affect a range of ornamental fish species including gouramis (Colisa spp. and Trichogaster spp.) (Anderson et al., 1993; Fraser et al., 1993; Paperna et al., 2001; Sudthongkong et al., 2002b), swordtails (Xiphophorus helleri) (Paperna et al., 2001), platies (Xiphophorus maculatus) (Paperna et al., 2001), mollies (Poecilia latipinna) (Paperna et al., 2001), angelfish (Pterophyllum scalare) (Rodger et al., 1997), orange chromides (Etroplus maculatus) (Armstrong and Ferguson, 1989), and African lampeye killifish (Aplocheilichthys normani) (Sudthongkong et al., 2002b). According to the International Committee on Taxonomy of Viruses, all megalocytiviruses are variants of a single species, ISKNV, and share >94% sequence identity within the major capsid protein (MCP) and the adenosine triphosphatase (ATPase) genes (Chinchar et al., 2005). However, a finer level of classification of strains is possible through sequence analysis and this can have epidemiological meaning (Go et al., 2006).

Prior to the outbreak in Murray cod, megalocytiviruses were not known to be established in Australia. However, a mortality event in dwarf gouramis imported to Australia from Singapore was consistent with such an infection (Anderson et al., 1993) and subsequently imported gouramis have been detected with megalocytivirus infection (Chong and Whittington, 2005). The megalocytivirus responsible for the disease in Murray cod was a minor variant of ISKNV, and closely related to another strain, dwarf gourami iridovirus (DGIV) (Go et al., 2006). An active trade in ornamental fish exists in Australia, involving the importation of large numbers of fish, including gouramis from south-east Asia. However, there is no trade in live mandarin fish. A survey of recently imported gouramis that died in retail aquarium shops in Sydney, Australia confirmed 22% to be PCR positive for megalocytivirus DNA (Go et al., 2006). The aim of this study was to test the hypothesis that megalocytivirus derived from imported gouramis was transmissible to and virulent in Murray cod.

2. Materials and methods

2.1. Source and husbandry of fish

2.1.1. Murray cod

Murray cod fingerlings (3–4 g, 6–7 cm total length) were purchased from a recirculating growout facility in north-eastern Victoria. Water was obtained from a bore. The fish were most likely to have been hatched in December 2003 at a commercial hatchery in central Victoria and were transferred to the growout farm in March. Fingerlings were transported to the University of Sydney in July 2004 and were placed into a 300 L circular plastic stock tank containing clay bricks to provide refuge. Biological filtration was by means of two Fluval 403 canister filters and flow rate was regulated to reduce turbulence. Fingerlings were fed daily with commercial floating native fish pellets (4 mm Grower, Ridley Aqua-feed).

2.1.2. Dwarf gourami

Apparently healthy adult dwarf gourami (Colisa lalia) (“Flame gourami” colour variety) (10 g, 6–7 cm total length) (n = 20) were purchased from a retail aquarium (Source B) (Go et al., 2006). The fish were recently imported from Southeast Asia, most likely from Singapore, and had been released from a mandatory 14 day quarantine period administered by the Australian Quarantine Inspection Service. Gouramis were fed daily a proprietary tropical fish flake (Wardley tropical fish flakes).

2.2. Transmission trials

All trials were conducted in 90 × 35 × 40 cm (120 L) glass aquarium. The bottom of each tank was covered in a thin layer of dark-coloured gravel in order to minimize reflections from the glass which disturb Murray cod. In
the case of the cohabitation trial, each tank was divided into two compartments using a perforated plastic fish-proof divider. Biological filtration for each tank was by means of a single Fluval 403 canister filter with flow rate regulated to reduce turbulence. Temperature was maintained at 27 °C (to mimic conditions experienced during the Victorian outbreak in February 2003) by a stainless steel aquarium heater with an external thermostat; temperature was verified daily using a calibrated laboratory thermometer. Lighting was controlled to provide ten hours of light and fourteen hours of darkness. Murray cod were acclimated for at least 1 week before use in trials while gouramis were allocated to treatment tanks upon arrival. Fish were inspected at least twice daily for morbidity and mortality and any moribund or dead fish were removed using a net specific for that tank. For intraperitoneal (IP) injection, fish were anaesthetized by immersion in benzocaine (40 mg/L) then injected in the ventral midline using a 1.0 mL tuberculin syringe. Controls to be injected with cell culture medium were injected before the fish to be given viral inoculum.

2.2.1. Intraperitoneal injection trial

Fifteen Murray cod fingerlings were randomly allocated to each of two tanks. One group was injected intraperitoneally (IP) with viral inoculum (see below) while the other was injected IP with cell culture medium. Fish remaining after 21 days were euthanased by overdose of benzocaine. This trial was repeated using the same experimental design.

2.2.2. Cohabitation trial

Fifteen Murray cod fingerlings were randomly allocated to one side of each of two partitioned tanks while 10 dwarf gouramis were randomly allocated to the other sides. In Tank A the gouramis were injected IP with cell culture medium while in Tank B gouramis were injected IP with viral inoculum. Fish remaining after 28 days were euthanased by overdose of benzocaine. No gourami negative control could be included as it was not possible to ensure that gouramis were free from iridovirus infection.

2.3. Viral inoculum

Homogenates were prepared from the abdominal viscera and/or eye and brain from four dwarf gouramis that were shown in a previous study to be PCR positive for megalocytivirus DNA (Go et al., 2006). The fish were from one aquarium shop (source A), thereby increasing the likelihood that only one strain of iridovirus was involved. Briefly, visceral tissue samples, each approximately 0.1 g, were removed from fish and stored in sterile tubes at −80 °C until required. After thawing, clarified tissue homogenates were prepared by grinding in cell culture media in a tube with a fitted pestle and vortexing with glass beads (Whittington and Steiner, 1993). Homogenates were stored at −80 °C until required. Two hours prior to inoculation, homogenates were thawed at 4 °C and 150–300 μL aliquots were taken from each homogenate, pooled, diluted approximately twofold using cell culture medium and then filtered using a 0.22 μm filter.

2.4. Tissue collection

Fish collected during daily inspections were immediately processed in preparation for histopathology and PCR with the aim of excluding megalocytivirus as a cause of morbidity and mortality. Some tissue from each fish was placed in 10% formalin for histopathology and other tissue was stored at −80 °C for PCR analysis. Eye and brain were used for PCR analysis in some cases to avoid residual inoculum that might have been present in the abdomen of fish injected IP. The abdominal viscera of all fish from the cohabitation trial were sampled for PCR analysis as was an eye from nine of the ten gouramis in Tank B in the cohabitation trial.

2.5. PCR analysis

Samples for PCR analysis were processed as described for the preparation of the homogenate for inoculation. DNA was extracted from the clarified homogenate using a High Pure Viral Nucleic Acid Kit (Roche) following the protocol included with the kit, with 200 μL of clarified homogenate used where the protocol called for serum, plasma or whole blood. 5 μL of extracted DNA from each individual was subjected to PCR amplification using primers C50/C51 which specifically amplify megalocytivirus DNA from the major capsid protein (MCP) gene, and reaction conditions as previously described (Go et al., 2006). Briefly, each PCR reaction was performed in a final reaction volume of 50 μL containing 45.0 μL of PCR mix and up to 5.0 μL of the appropriate DNA sample. Each PCR mix consisted of PCR-grade water to a final reaction volume of 45 μL, 25 μM of each deoxynucleotide triphosphate (dATP, dTTP, dGTP, dCTP), 2 μM of each oligonucleotide primer, 10× PCR buffer (50 mM KCl, 10 mM Tris–HCl pH8.6, 2.5 mM MgCl₂ with addition of 10 mM beta-mercaptoethanol at time of preparation of the PCR mixture) and 2 units of Taq DNA.
polymerase (Marsh et al., 2002). Reactions were performed using a 96 place thermal cycler (Corbett Research, Sydney), with the following profile: 1 cycle of denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min. The reactions were then cooled to 4 °C. A modified hot start procedure was used whereby samples were not loaded onto the thermal cycler until the block had reached 94 °C during the initial denaturation cycle (Marsh et al., 2002). PCR products were separated by gel electrophoresis on a 2% agarose gel containing 0.003% ethidium bromide, and compared against molecular size marker number VIII (Roche).

2.6. DNA sequencing

To verify that transmission of the same iridovirus had occurred, DNA sequencing was undertaken of the complete MCP and ATPase genes of megalocytivirus present in the viral inoculum, Murray cod from the IP injection trial and both Murray cod and gourami from cohabitation Tank A. The sequencing protocol and primers used are described elsewhere (Go et al., 2006). Sequencing was carried out in both the forward and reverse directions for verification. Predicted amino acid sequences were derived with the reading frame determined by alignment with known coding regions of homologous genes in GenBank, using ETRAN-SLATE through the Australian National Genomic Information Service http://www.angis.org.au/html/bioinformatic/webangis.html). Alignments were made between sequences and homologies were calculated using HOMOLOGIES, with sequence gaps excluded from calculations, using only those regions common to all viruses in the alignment.

2.7. Histopathology

Transverse sections of each fish approximately 2–3 mm thick were embedded in paraffin, sectioned at 5 um and stained with haematoxylin and eosin. Samples that contained lesions with characteristic hypertrophied cells in one or more tissues were classified as positive (Gibson-Kueh et al., 2003).

2.8. Transmission electron microscopy

Transmission electron microscopy (TEM) was performed on tissue from one fish that contained representative viral inclusions visible in light microscopy. Briefly, tissue was excised from the selected paraffin block, dewaxed in xylene, rehydrated in 100 per cent acetone and placed through a graded ethanol series. Tissue was then fixed in osmium tetroxide and stained in uranyl acetate, dehydrated through a graded ethanol series and transferred to acetone before being embedded in Spur’s resin. Ultrathin 80 nm sections were cut and stained with uranyl acetate and lead citrate and examined using a Philips 208 transmission electron microscope.

3. Results

3.1. Intraperitoneal injection

Mortality commenced 8–9 days post inoculation in Murray cod injected with PCR-positive gourami filtrates...
and only one fish survived to day 21 (Figs. 1 and 2, Table 1). Fish began to darken, had open mouth ventilation and rapid opercula movements approximately 24 h prior to death. These fish remained at the bottom of the tank when approached, and did not feed whereas control fish swam towards the surface and fed actively. Fish assumed a position of lateral recumbency immediately prior to death in the few cases where this could be observed.

One control fish died during the trial period due to intraspecific aggression; it had lost an eye and its fin rays were damaged. Four controls were euthanased in benzocaine on day 13 to enable direct comparisons to be made with treatment fish that had died, and the remaining 25 were euthanased 21 days after inoculation.

In trial 1, PCR was performed on the abdominal viscera from all fish and on the eye and brain of some. In the second trial PCR testing was based on eye samples only from fish injected with virus and both eye and abdominal viscera of the controls. All 29 affected fish that had been injected with the viral inoculum were PCR positive from all samples tested while none of the controls were PCR positive.

The tissues of the majority of Murray cod that died contained hypertrophied cells consistent with

![Fig. 2. Cumulative mortality in gourami and Murray cod kept in the same tank in the cohabitation trial. Diamonds, gourami, G; squares, Murray cod, MC. Tank A, gourami injected with cell culture media; Tank B, gourami injected with viral inoculum.](image)

Table 1
Mortality and laboratory results for Murray cod in the intraperitoneal transmission experiments

<table>
<thead>
<tr>
<th>Trial</th>
<th>Group</th>
<th>n</th>
<th>21 day outcome</th>
<th>PCR</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ve</td>
<td>−ve</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>15</td>
<td>Dead</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Live</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Viral inoculum</td>
<td>15</td>
<td>Dead</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Live</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>15</td>
<td>Dead</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Live</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Viral inoculum</td>
<td>15</td>
<td>Dead</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Live</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Controls were injected with cell culture medium. +ve, positive; −ve, negative.

a PCR on abdominal viscera ± eye/brain.

b PCR eye and abdominal viscera.

c PCR on eye only.
megalocytivirus infection (Table 1, Fig. 3). Iridoviral virions 125–130 nm in size (measured vertex to vertex) were detected in a representative fish in which inclusions were visible by light microscopy (Fig. 4).

3.2. Cohabitation trial

Mortality in Murray cod reached 35–40% by day 28. The pattern of mortality was consistent with an infectious process rather than a response to handling fish or trauma (Table 2, Fig. 2). Of the 11 Murray cod that died, 55% were PCR positive while 27% were histologically positive. Of the Murray cod that survived to 28 days, 32% were PCR positive but none had histological evidence of megalocytivirus infection. None of the Murray cod that died had noticeable premonitory clinical signs, except for the last fish to die in Tank A, which was darkened, reluctant to move from the bottom of the tank and had respiratory distress on the day prior death.

One gourami died in Tank A on day 13. This fish had pale colouration consistent with a published account of natural iridoviral infection in this species (Sudthongkong et al., 2002b), constant opercular movement and was gasping at the surface of the water on the day immediately prior to death. Its tissues were PCR positive and it had histological findings consistent with megalocytivirus infection. Two other gourami in Tank A were PCR positive. Three gourami died in Tank B and apart from mild lethargy there were no premonitory signs. The tissues of two were PCR positive but megalocytivirus inclusions were not detected histologically (Table 2).

3.3. DNA sequencing

DNA sequencing confirmed 99.9%–100% homology of MCP nucleotide sequences, and 99.8%–100% homology of amino acid sequences between DGIV-2004...
(GenBank Accession AY989901), ISKNV (AF371960), MCIV (AY936203), the viral inoculum obtained from dwarf gourami for intraperitoneal inoculation, the virus present in media-injected dwarf gourami used for cohabitation and the virus present in Murray cod infected experimentally by intraperitoneal injection or cohabitation. Similarly for ATPase there was 99.9%–100% homology of nucleotide sequences, and 100% homology of amino acid sequences between these same viruses (DGIV-2004, AY98902; ISKNV AF371960; MCIV AY936204). Nucleotide homologies for MCP were determined over 1362 bp and amino acid homologies were determined over 454 residues, while the corresponding figures for ATPase were 1705 bp and 239 residues.

4. Discussion

The megalocytivirus present in the viral inoculum prepared from dwarf gourami was identified as a strain of the species ISKNV. It was highly virulent for Murray cod fingerlings following intraperitoneal injection. Most fish died within 21 days and were positive in a specific PCR assay for megalocytivirus DNA. Basophilic hypertrophied cells characteristic of megalocytivirus infection were found in 23 of 29 fish and a representative lesion examined by electron microscopy contained icosahedral virions. There were qualitatively similar findings but there was a lower level of mortality when the virus was transmitted to Murray cod via water by cohabitation with dwarf gourami. DNA sequencing of two viral genes was used to show that the same virus was present in dwarf gourami and experimentally infected Murray cod. These results confirmed both infectivity and virulence of DGIV for Murray cod.

Tissues from 50% of the fish that died contained megalocytivirus DNA demonstrable by PCR and typical histological lesions were seen in many fish. The lack of positive histological and PCR results in all fish may have been due to the acute nature of the mortalities which provided insufficient time for the histological lesions to develop and/or the incomplete range of tissues examined in each test, so that lesions and tissues that contained virus were not examined. The analytical sensitivity of the PCR assay is not known and its susceptibility to inhibition when applied to clinical samples is also uncertain. As some of the surviving Murray cod fingerlings were also PCR positive, it is possible that 28 days was an insufficient period for clinical disease to develop in all of the fish that became infected.

The disease reported from Murray cod from the Victorian farm in 2003 (Lancaster et al., 2003) and that induced experimentally in the same species in this study were not distinguishable clinically or pathologically. Some of the dwarf gourami used in these experiments had pre-existing DGIV infection evidenced by occurrence of clinical disease after their acquisition from retail premises, positive PCR and histopathology findings, and by transmission of DGIV to Murray cod in Tank A in the cohabitation experiment. There was also indirect evidence of resistance of some gouramis to DGIV. In Tank B, in which gouramis were injected IP with viral inoculum, there was low mortality, few gouramis tested positive in PCR, and none of the fish had histopathological lesions to indicate viral replication. Reasons for this apparent resistance include prior exposure to DGIV or an antigenically related megalocytivirus producing acquired immunity, or innate resistance. This is consistent with reports of megalocytivirus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species</th>
<th>N</th>
<th>Number of fish</th>
<th>28 day outcome</th>
<th>PCR²</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ve</td>
<td>−ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Tank A–gourami injected with cell culture media</td>
<td>Dwarf gourami</td>
<td>10⁴</td>
<td>Dead</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Murray Cod</td>
<td>15⁴</td>
<td>Live</td>
<td>9</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Tank B–gourami injected with viral inoculum</td>
<td>Dwarf gourami</td>
<td>10⁵</td>
<td>Dead</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Murray Cod</td>
<td>15⁵</td>
<td>Live</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

+ve, positive; −ve, negative.

a PCR on abdominal viscera.
b PCR on abdominal viscera and eye.
c 1 fish not examined.
in dwarf gouramis (Anderson et al., 1993; Sudthongkong et al., 2002b) and indeed, Sudthongkong et al. (2002a,b) when attempting to experimentally transmit African lampeye iridovirus (ALIV) by injection chose to use pearl gourami (Trichogaster leeri) in preference to dwarf gourami on the basis of the prevalence of iridovirus infection among dwarf gourami. Incomplete mortality (50%) was observed in these presumably naïve pearl gourami (Sudthongkong et al., 2002b). Furthermore, dwarf gourami kept in the same ponds as other gourami species with iridoviral disease did not develop signs of infection (Paperna et al., 2001). Thus there may be innate resistance to megalocytivirus in a number of gourami species.

The virus that caused the epizootic in Murray cod in Victoria in 2003 was a strain of ISKNV, the type species of megalocytivirus found in mandarin fish in China and was named Murray cod iridovirus (MCIV) (Go et al., 2006). DGIV and MCIV were almost indistinguishable from ISKNV, suggesting a common recent origin for all three viruses. About 6% of the 9 million live ornamental fish imported annually into Australia are gouramis, whereas no live mandarin fish are imported (Kahn et al., 1999; Love et al., 2004). This implicates gourami as the source of DGIV and related viruses in Australia. DGIV has been detected in imported gourami in Australia on many occasions (Chong and Whittington, 2005; Go et al., 2006). These results support previous claims of a potential link between the international trade in ornamental fish and the development of disease and mortality in farmed fish species (Hedrick, 1996; Hedrick and McDowell, 1995). The confirmation of transmissibility to and virulence of DGIV for Murray cod has significant implications for aquaculture, ecological management and quarantine policy.

The ability of DGIV to transmit from gourami to Murray cod through water indicates that the culture of the two species in a common water source should be avoided. Various species of gouramis are cultured on fish farms in different states of Australia (Beesley and O’ Sullivan, 2000; O’ Sullivan and Ryan, 2001) as are Murray cod (Brown et al., 1997). However, quarantine policy was informed by the belief that production of gouramis in Australia was limited to aquaria and by the assumption that gouramis were “...not associated with significant pathways by which infected fish may enter the natural environment” (Kahn et al., 1999). At least one farm in Queensland produces blue (three-spot) gouramis (Trichogaster trichopterus) in open ponds, as well as producing fingerlings of aquaculture species (Anon, 2006). Should the endemic breeding population of gouramis be infected with DGIV derived from imported stock, this situation would provide a potential means for transmission of DGIV to other farmed fish species, and spread of the infection by escape of fish or discharge of waste water into waterways, and by commercial translocation of fish.

Similarly, free-living populations of three-spot gourami in the Burdekin region of northern Queensland, Australia (Raadik, 2003) could potentially act as a reservoir for amplification of megalocytivirus for subsequent dispersal to the local aquatic environment. Queensland is currently the only state known to have free-living gourami that have escaped from the pet industry (Lintemans, 2004). However, other species may be susceptible to megalocytivirus infection and act as carriers. For example, the mosquito fish (Gambusia affinis) is widely distributed across Australia (McDowall, 1996), and is closely related to poecilid aquarium species such as swordtails (X. helleri) and mollies (P. latipinna), which are known to be susceptible to infection by megalocytiviruses (Paperna et al., 2001).

It is unclear whether DGIV has become established in Australia and surveys will be required of free-living gouramis and commercial breeding gouramis, all of which are derived from imported stock. There is also strong indication for research to test the susceptibility of a range of fish species to DGIV. This would provide invaluable information for aquaculture and ecological management of escaped ornamental species, farmed fish and native species. Strains of DGIV are known to produce mortality in at least two species in the Family Percichthyidae: mandarin fish, S. chuatsi, and Murray cod. It is highly probable that other members of this family would be susceptible. Approximately sixteen species of fish belong to the Family Percichthyidae, of which nine are found in Australia (Allen et al., 2002). Many are listed as vulnerable or endangered (Morris et al., 2000) while a number are important aquaculture or fisheries species (Brown et al., 1997).

Feeding ‘trash fish’ derived either from fisheries sources or the ornamental fish industry to farmed fish should be discouraged, given the possibility of ingestion of megalocytivirus. A similar route of transmission has been implicated in the development of megalocytivirus disease due to ISKNV in China (He et al., 2002). However, further work needs to be carried out in relation to transmission via ingestion.

Further studies are needed to determine the properties of DGIV including persistence in the environment and susceptibility to heat, ultraviolet radiation, ozone and common chemical disinfectants. These studies will inform disease response recommendations but first
will require optimization of cell culture. Currently, almost nothing is known of the pathogenesis of diseases caused by this virus in any species of finfish. This would limit vaccine development. Vaccination is available for RSIV in marine aquaculture in Japan (Nakajima et al., 2001), but this virus is not closely related to DGIV at the molecular level (Go et al., 2006) and there may be little antigenic cross reactivity.

Gourami imported into Australia are subject to mandatory quarantine for 14 days. However, it is clear that this period is insufficient as it does not prevent subclinically infected individuals from entering the retail trade. Indeed DGIV-infected gourami have been confirmed in two aquarium shops in Sydney (source A, source B) after release from quarantine administered by the Australian Quarantine Inspection Service (Go et al., 2006). Refinements to quarantine policy will be critical in preventing future entry of this group of viruses and other aquatic pathogens into Australia (Chong and Whittington, 2005).

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