Aspects of the ornamental fish industry: Implications

ornamental fish

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Recently viral agents have emerged as the source of significant disease and mortality in fish worldwide. It has long been recognised that the movement of ornamental fish through exporting and importing practices provides a transmission pathway for the introduction and establishment of exotic viral pathogens. Aspects of ornamental fish aquaculture are reviewed with a focus on the disease risks associated with the importation of live ornamental fish in Australia. The viral agents *Megalocytivirus* and *Cyprinid herpesvirus-2* (*CyHV-2*), which represent significant exotic pathogens to Australia, are detailed through the use of case studies which confirm that the disease risks posed by the importation of ornamental fish are genuine. These cases highlight the need for investigation to determine the prevalence of these viral pathogens in post-quarantine situations such as aquatic retail outlets which has important implications for policy and management. The potential for the use of molecular diagnostic tools such as standard and quantitative polymerase chain reaction (PCR) for the detection of exotic viral pathogens is proposed as a way of addressing the apparent inadequacies of the current Australian quarantine program whereby the occurrence of inadvertent disease release is unacceptable.

Keywords: Fish, ornamental, virus, polymerase chain reaction

Introduction

Due to the growing popularity and wide international culture of keeping ornamental fish, Australian aquatic retail outlets import large numbers of a wide range of ornamental fish. As a result of this pattern of trade and despite a mandatory quarantine period many incursions of disease of fish have been identified, of which ornamental fish have been shown to provide a transmission pathway for disease (Stephens *et al.* 2004, Go *et al.* 2006), in particular viral agents. Viral diseases therefore represent a significant threat to not only the Australian aquaculture industry but also to Australian native fish species.

The objective of this review is to discuss the Australian ornamental fish industry and current quarantine policy and present the numerous risks and concerns associated with the importation of ornamental fish in this country. Finally, a review of current methods of viral pathogen detection is necessary as it highlights the need and potential of laboratory techniques such as PCR for the recognition of exotic viral pathogens in populations of imported ornamental fish.

Aquaculture and sources of ornamental fish

Aquaculture is generally considered to be the farming or husbandry of aquatic organisms for commercial purposes (Tlusty 2002) and as such there are many different forms of aquaculture. The use of the word farming not only implies that there is deliberate human intervention in the production cycle but also that there is ownership of the stock being cultivated (Tlusty 2002, Lucas and Southgate 2003). Currently there exists a worldwide trend in aquaculture whereby the majority of aquaculture occurs in the form of food production. Nevertheless ornamental fish production, which is the production of animals for the aquarium hobbyist trade (Tlusty 2002), is increasingly becoming an important component of the aquaculture industry.

Ornamental fish keeping is a popular hobby worldwide with an estimated 12-14% of the population of Australia participating in the hobby (Anon 2006). The appeal of an aquarium in which little space is required and a wide range of aquarium fish in an array of different shapes and colours is available, according to enthusiasts, creates a relaxing, interesting and enjoyable hobby. This along with the fact that

there are many perceived therapeutic and social benefits of keeping ornamental fish (Harper 2007) ensures that enthusiasts continue to make large investments in all aspects of ornamental fish keeping.

Due to an increase in consumer demand in local and international markets the production of ornamental fish is steadily increasing. Aquaculture of freshwater ornamental fish is typically conducted in closed tanks or ponds, usually in conjunction with indoor facilities that house numerous small tanks. Generally, facilities for the production of aquarium fish are small compared to major food-fish production operations (Tlusty 2002, Livengood and Chapman 2007). Although the majority of ornamental fish in the aquarium trade are of freshwater origin and farm-raised, it is estimated that over 90% of marine ornamental fish are from wild-caught fisheries (Livengood and Chapman 2007). An excess of 1450 species of marine fish are traded worldwide with Indonesia and the Philippines among the main suppliers (Whittington and Chong 2007). Aquaculture production of ornamental fish, under proper developmental conditions, has the potential to positively impact the global economy. Furthermore, as many of the fish in the industry originate from developing countries, the ornamental fish industry therefore represents a major source of economic benefit to developing countries (Tlusty 2002).

Ornamental fish industry

The ornamental fish industry, which includes a vast array of animal species, is made up of approximately 2000 species of fish, invertebrates, crustaceans, molluscs and live rock (Livengood and Chapman 2007). As fish represent the majority of species traded worldwide, when referring to the ornamental fish industry this paper will be concerned with fish only.

Fish aquaria are displayed in many different situations including homes, offices and public areas as keeping ornamental fish continues to be a popular hobby worldwide. Since 1985 the ornamental fish industry has experienced an average annual growth rate of 14% (Whittington and Chong 2007). O'Sullivan *et al.* (2008) estimates the retail value of the ornamental fish industry to be worth between AU$3 billion and AU$4.5 billion

Extensive trade in imported freshwater and marine fish species as well as locally caught or reared fish is characteristic of the ornamental fish industry. As such within this industry there is a well-established network of trade people, comprising importers, wholesalers, breeders, retailers and consumers or hobbyists (Kahn *et al.* 1999, O'Sullivan *et al.* 2008) (FIG. 1). The ornamental fish trade is primarily driven by consumer demand with overall markets in the United States of America accounting for 60% of the global ornamental fish production, while markets in Western Europe, Japan, Taiwan and Australia account for the remainder (Tlusty 2002).

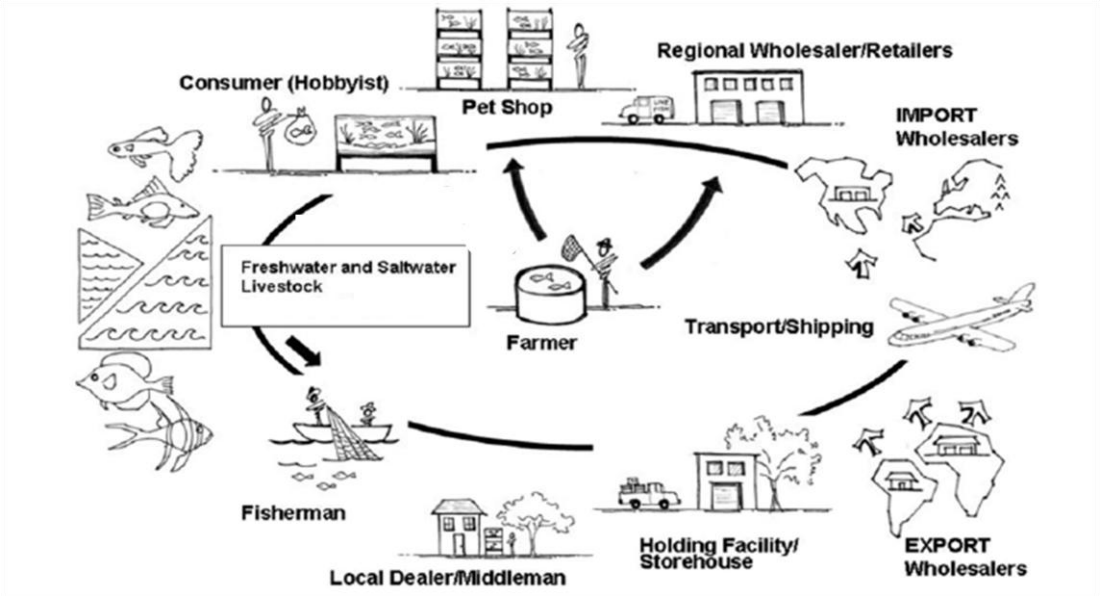

Figure 1 Schematic representation of the typical distribution and trade process of ornamental fish. The number of business intermediaries in the distribution chain depends on the species, origin, abundance, popularity and proximity to markets (Livengood and Chapman 2007)

The Australian ornamental fish industry

In Australia the ornamental fish industry is represented by the Pet Industry Joint Advisory Council. The lack of detailed assessment of the ornamental fish industry in Australia regarding its volume and value is exemplified by the limited statistical information available on imports and exports of ornamental fish within the country (Kahn *et al.* 1999). Although this is the case, industry members value the ornamental fish industry, including accessories such as food and tanks, in Australia at AU$220 million annually (O'Sullivan *et al.* 2008) with Australia importing 17.7 million ornamental fish in 2006 – 2007 (O'Sullivan *et al.* 2008). Imports from major sources such as Asia account for 40% of Australia's ornamental fish trade (Kahn *et al.* 1999). These Australian industry figures are expected to grow with the increasing popularity of the hobby of keeping ornamental fish, which has been largely facilitated by advances in fish husbandry and aquarium equipment technology so that there are now millions of enthusiasts worldwide.

The majority of ornamental fish imported to Australia consist of over 600 genera within the classes Actinopterygii (Osteichthyes) and Chondrichthyes (Kahn *et al.* 1999). Goldfish (*Carassius auratus*) which constitute 22% of imports represent the greatest proportion of species of imported ornamental fish in Australia (Kahn *et al.* 1999). Although a large portion of the ornamental fish industry in Australia is derived from imported stocks, as the market grows local ornamental fish production is increasing. More recently due to a gain in popularity there has been an interest in the production of Australian native freshwater species such as rainbowfish (*Melanotaenia spp.*) and juvenile food fish such as barramundi (*Lates calcarifer*) for the aquarium market (Kahn *et al.* 1999, Love and Langenkamp 2003). Aquaculture production of ornamental fish species in Australia occurs mostly in New South Wales, Victoria, Queensland and Western Australia, whereby in 2001 – 2002 it was estimated that 6.9 million ornamental fish were produced at a value of AU$4.5 million in these states (Love and Langenkamp 2003).

Currently the production of marine ornamental fish using aquaculture based methods is more costly than harvesting marine ornamental fish from the wild. As such, within Australia, marine ornamental fish are harvested from the wild with this practice mainly occurring in Queensland in places such as the Great Barrier Reef (Kahn *et al.* 1999). Due to the importance of sustainability, wild harvesting of ornamental fish in Australia is highly regulated. To ensure longevity of the ornamental fish industry in Australia it is important that the industry continues to produce consistently high quality fish with superior heath status and that within the industry imported ornamental fish are and remain disease-free.

Quarantine policy and legislation

As a way of ensuring the health status of imported ornamental fish and minimising the spread of disease quarantine measures are necessary. In Australia importation of live ornamental fish is regulated by the Australian Quarantine and Inspection Service (AQIS), which aims at reducing the risk posed to the Australian agriculture industries and environment by exotic pests and diseases. The import conditions for live freshwater and marine ornamental fish are available respectively at (last updated April 30, 2008):

http://www.aqis.gov.au/icon32/asp/ex_casecontent.asp?intNodeId=8192061&intCommodityId=6114&Types=none&WhichQuery=Go+to+full+text&intSearch=1&LogSessionID=0

http://www.aqis.gov.au/icon32/asp/ex_casecontent.asp?intNodeId=8192194&intCommodityId=6115&Types=none&WhichQuery=Go+to+full+text&intSearch=1&LogSessionID=0

There have been many reviews and risk assessments relating to the importation of live ornamental fish in Australia (Humphrey 1995, Anon 1999, Kahn *et al.* 1999, Chong and Whittington 2005, Anon 2006) which provide information on whether Australia's biosecurity objectives are being met in regard to quarantine of ornamental fish. Some have concluded that current quarantine measures provide an unacceptably low level of protection thus providing a case for revision (Chong and Whittington 2005, Whittington and Chong 2007). The occurrence of disease in post quarantine populations of ornamental fish supports this conclusion and will be further discussed in detail.

Australia has one of the most stringent standards for the importation of ornamental fish (Whittington and Chong 2007), which includes pre-border health certification and a mandatory 1 – 3 week quarantine period in quarantine approved premises. Despite these measures many disease and environmental risks

have been recognised as a result of the trade in live ornamental fish, some of which have proven to be genuine (Stephens *et al.* 2004, Go *et al.* 2006).

Risks associated with current ornamental fish trading practices

In 2006 there were 22 species of imported ornamental fish in the wild with established breeding populations in Australia (Lintermans 2004). This proves the existence of a direct pathway for imported ornamental fish to come into contact with domestic or native fish species which in turn may be potentially exposed to exotic pathogens. This not only has important implications for conservation but also raises concerns regarding the introduction and establishment of pest species. Australia possesses natural environments which contain unique and diverse populations of indigenous fishes. The need to maintain this biodiversity necessitates research into examining the health status of imported ornamental fish in Australia and developing effective diagnostic tests in order to detect known or emerging exotic aquatic animal pathogens.

An overwhelming majority of emerging diseases originate and arise from wild reservoir hosts and are spread globally by man (Whittington and Chong 2007). Exotic disease introduction through the transmission pathway of imported ornamental fish represents a major threat to wild fish populations and the ornamental and foodfish aquaculture industries in Australia (Whittington and Chong 2007). This problem is intensified by the fact that many aspects of the current quarantine policy, including pre-border policies, duration of quarantine and inspection and surveillance during quarantine, have been recognised as providing inadequate protection against the possibility of exotic disease introduction (Chong and Whittington 2005).

The low rate of disease diagnosis and monitoring of ornamental fish in Australian quarantine premises is indicative of a lack of surveillance of ornamental fish pathogens entering Australia (Chong and Whittington 2005). Visual inspection is insufficient at differentiating healthy fish from those carrying a pathogen in the latent or carrier state, which is common to both viral and bacterial infections. Consequently a review of Australian ornamental fish import risk management recommended that laboratory testing be carried out as an effective way of detecting exotic pathogens and limiting the occurrence of inadvertent release of exotic pathogens by quarantine (Chong and Whittington 2005).

Upon examination of the current conditions for the importation of freshwater and marine ornamental fish, it is apparent that these conditions are less stringent than the policy for any other live animal imports. This can be seen in terms of the number of species permitted, the number of countries of origin permitted and the overall knowledge of disease and prevalence in these countries, as well as the level of disease diagnosis whilst in quarantine (Chong and Whittington 2005). This inequality oddly suggests that there is a greater tolerance for the risk of disease introduction from fish compared to terrestrial animals.

Viral Disease

Historically the term “virus” was used to describe a poison or soluble noxious agent (Post 1983), however it is now known that viruses are particulate with a genome consisting of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Unlike bacteria or fungi, viruses do not possess a nuclei or organelles used for maintenance and as such the single viral particle, or virion, depends on the host cells synthesising structures for replication (Post 1983). The nucleic acid of the virion is enclosed in a protein coat called the capsid, which is organised in aggregates of structural units known as capsomeres, forming a nucleocapsid. Depending on the type of virus the nucleocapsid of the virion may be surrounded by a lipid containing envelope, however if the envelope is not present the virus is termed ‘naked’ (Post 1983). In terms of shape, viruses which infect fish are either spherical, having an icosahedral (20-sided) shaped nucleocapsid, or rod-shaped, having helical nucleic acid symmetry (Post 1983).

The source of outbreaks of viral diseases is usually from farmed or wild carriers and as previously discussed the movement or translocation of infected species can aid in the spread of viruses throughout a particular watercourse. Transmission of viral disease can occur horizontally between fish, whereby skin abrasions, gills and the gut represent the main routes of infection, or vertically through the egg of infected broodstock to their offspring (Brown 1993). Additionally some viruses may be transmitted horizontally between species. Go and Whittington (2006) showed that the virus known as dwarf gourami

iridovirus (DGIV) was able to be transmitted from dwarf gourami (*Colisa lalia*), an ornamental fish species, to Murray cod (*Maccullochella peelii peelii*) through water.

Many different viruses have been identified in fish with some having great economical importance. Such viruses can be found in the Family *Iridoviridae*, which over the last two decades have emerged as important pathogens in aquaculture having been the cause of epizootics which have occurred worldwide (Go *et al.* 2006). Viruses in this family which affect fish species farmed for food include RSIV, infectious spleen and kidney necrosis virus (ISKNV), rock bream iridovirus (RBIV) and grouper sleepy disease virus (GSDV). Additionally African lampeye iridovirus (ALIV) and DGIV are iridoviruses which affect ornamental fish species (Go *et al.* 2006). Due to sequence homologies between these viruses and the fact that they share similar clinical signs and pathology this group of viruses are now recognised within the genus *Megalocytivirus* (Go *et al.* 2006). *Megalocytiviruses* are composed of large double-stranded DNA, are approximately 120-350nm in size and possess icosahedral symmetry.

Viruses in the Family *Herpesviridae*, which include carp pox herpesvirus (*Cyprinid herpesvirus-1*, *CyHV-1*), herpesviral haematopoietic necrosis virus (*Cyprinid herpesvirus-2*, *CyHV-2*) and koi herpesvirus (*Cyprinid herpesvirus-3*, *CyHV-3*), also represent important pathogens in aquaculture. These viruses are composed of a linear, double-stranded DNA genome which is packaged within an icosahedral capsid surrounded by a host-derived envelope (Waltzek *et al.* 2005). Due to a number of disease outbreaks in goldfish throughout the world, including Australia, which have been associated with *CyHV-2* (Jung and Miyazaki 1995, Stephens *et al.* 2004, Goodwin *et al.* 2006b, Jeffery *et al.* 2007) there is a need for further research into the distribution of this virus, in which its spread is likely to have been facilitated by the trade in ornamental fish (Whittington and Chong 2007).

Viral disease incursions associated with imports

As previously outlined, the potential for the importation of diseases through the trade in live ornamental fish is internationally recognised (Mouton *et al.* 2001). Although this is the case the role of ornamental fish in the translocation and establishment of exotic viral pathogens in both ornamental and non-ornamental fish species has received limited attention (Whittington and Chong 2007). Nevertheless the following case studies outlining viral disease incursions in Australia which have been shown to be associated with imported ornamental fish provide direct evidence that the risk for introduction of exotic infectious diseases discussed in previous chapters are genuine. Consequently these cases, and indeed many others, are not only a large concern to aquaculturists but also to conservationists.

Case study 1: *Gourami iridovirus*

Iridoviruses have been implicated as the cause of severe disease, mortality and economic loss in Australia in a range of fish species including ornamental fish, farmed food fish and wild fish (Langdon *et al.* 1986, Plumb *et al.* 1996, Gibson-Kueh *et al.* 2003, Jeong *et al.* 2008). In 2003 in Victoria, Australia a mass mortality event occurred in intensively farmed Murray cod (FIG. 2), whereby over a period of several weeks 90% of 10 000 fingerlings died (Lancaster *et al.* 2003). Clinical signs included inappetance, followed by lethargy and death within 4 to 7 days.

In Australia there are three endemic iridoviruses which affect fish namely, epizootic haematopoietic necrosis virus (EHNV) known to infect wild redfin perch (*Perca fluviatilis*) and farmed rainbow trout (*Oncorhynchus mykiss*), Bohle iridovirus known to infect amphibians and lymphocystis disease virus (LDV) (Go *et al.* 2006) which was the first iridoviral disease described in marine and reshwater species of fish (Gibson-Kueh *et al.* 2003). Based on a negative result from an immunoperoxidase test incorporating rabbit polyclonal anti-EHNV antiserum, the absence of reports of infection of Murray cod with Bohle iridovirus (Speare and Smith 1992, Moody and Owens 1994) and viral morphology non-indicative of LDV (FIG. 2), Lancaster *et al.* (2003) established the cause of Murray cod deaths as an iridovirus which was not endemic to Australia.

A study by Go *et al.* (2006) which involved sequencing of viral genes from the Murray cod revealed that compared with viral genes from imported Asian dwarf gouramis (FIG. 2) which had died in Australian aquatic retail outlets, 99.95% of the sequences were identical. These two viruses, Murray cod iridovirus (MCIV) and DGIV, therefore represent a single species within the *Megalocytivirus* genus and due to this large homology are likely to share a common geographic origin. Consequently the origin of infection was found to be linked with the trade in ornamental fish in Australia (Go *et al.* 2006).

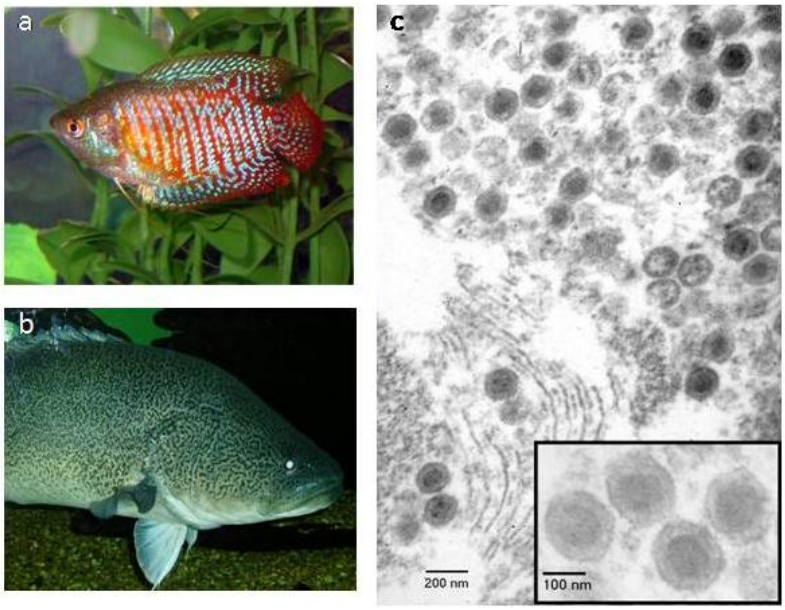

Figure 2 a) A dwarf gourami (*Colisa lalia*); b) a Murray cod (*Maccullochella peelii peelii*) which is a species farmed for human consumption but threatened where it exits in the wild; c) electron micrograph of Murray cod spleen showing naked icosahedral virions with a mean diameter of 148 +/- 8nm (images courtesy of Professor Richard Whittington)

Case study 2: *Herpesviral haematopoietic necrosis virus*

In 2004 in Perth, Western Australia 1 of 14 goldfish purchased from a wholesale supply company for a private collection died after 4 weeks (Stephens *et al.* 2004). Following death an investigation by necropsy, histological and transmission electron microscopy examination occurred, whereby severe, diffuse necrosis of haematopoietic tissue in the spleen and kidney was found, along with intranuclear viral particles with morphologic characteristics typical of herpesvirus (FIG. 3). The authors of this case report therefore concluded that due to the presence of a pathogen similar to herpesvirus in a goldfish with severe haematopoietic necrosis the origin of infection was indicative of herpesviral haematopoietic necrosis virus caused by the agent *CyHV-2*, an agent which had not previously been reported in Australia.

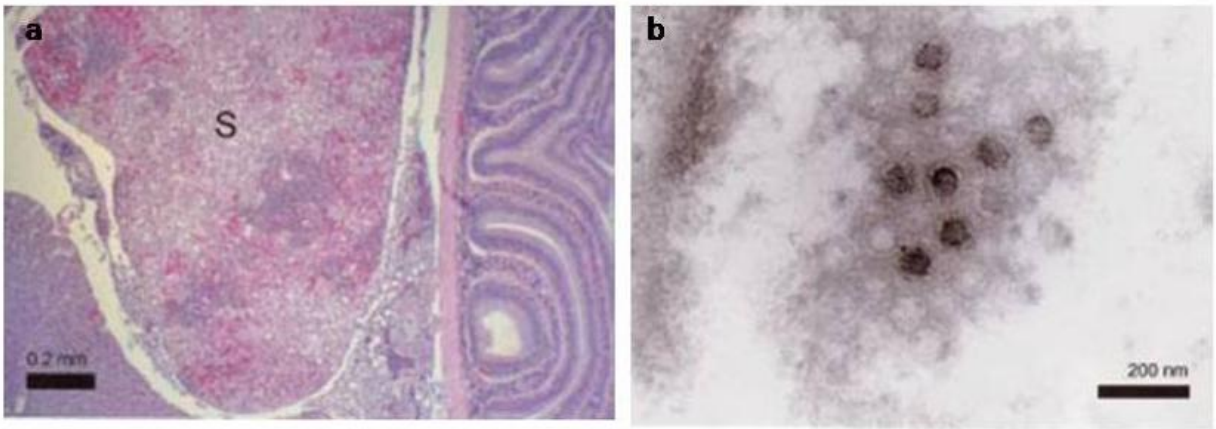

Figure 3 a) A section of the spleen (S) showing severe, diffuse necrosis of haematopoietic tissue of the infected goldfish (*Carassius auratus*); b) an electronmicrograph of an enlarged nucleus in the spleen. Icosahedral viral nulceocapsids morphologically similar to herpesviruses are demonstrated (Stephens *et al.* 2004).

In contrast to the case of gourami iridovirus, this present case was not an epizootic resulting in substantial mortality, with the pattern of mortality being indicative of a carrier fish developing overt disease following stress (Stephens *et al.* 2004). This suggests that although no epizootics have been reported they may have occurred but failed to be fully investigated (Stephens *et al.* 2004). Nevertheless this case indicates the introduction of herpesviral haematopoietic necrosis virus to Australia and highlights the further potential for the introduction of other exotic pathogens with imported ornamental fish.

Methods of viral disease detection

Currently prevention of exposure of fish to the virus is the most effective method of controlling diseases caused by viruses. As such there is a need for health management programs that require appropriate methods for the detection of the presence of exotic viral pathogens in populations of imported ornamental fish. Recent advances in infectious disease diagnosis in human, plant and terrestrial animal species are increasingly being applied to the health and management of economically important aquatic species (Bowers *et al.* 2008). This indicates the potential for the use of molecular diagnostic tools for not only small scale on-farm surveillance but also for large scale regulation of aquatic animal trade between countries.

Diagnosis of viral outbreaks can be achieved using a number of different methods. Disease diagnosis can be based upon clinical observations, histological findings and demonstration of viral particles using electron microscopy in tissues of infected fish. Almost all reported cases of herpesviral haematopoietic necrosis virus have been diagnosed this way (Groff *et al.* 1998, Chang *et al.* 1999, Stephens *et al.* 2004), however Goodwin *et al.* (2006a) report that these methods could result in ambiguous identification of the virus. Detection of viral disease can also rely on the isolation of viruses from infected or suspect fish using established fish cell lines. Upon isolation the virus must then be identified or confirmed which further involves neutralization tests with specific polyclonal antisera or enzyme immunoassays with monoclonal antibodies, processes which can take 1 – 4 weeks (Williams *et al.* 1999).

Due to the time constraints and difficulty of isolating viruses, whereby currently there have been limited reports of the successful propagation of *CyHV-2 in vitro* (Jung and Miyazaki 1995, Jeffery *et al.* 2007), molecular diagnostic techniques that can be applied directly to fish tissues such as polymerase chain reaction (PCR) assays are increasingly becoming incorporated into fish health management programs. Detection of pathogen nucleic acids by PCR can be faster (results are available within hours), more sensitive and specific than culture methods (Kerr and Cunningham 2006) and as such there is much interest in developing and optimising both standard and quantitative PCR assays for the detection and identification of fish viruses of significance.

Standard PCR

Individual PCR assays for detecting and identifying fish viruses such as *Megalocytivirus* and *CyHV-2* have been developed (Williams *et al.* 1999, Go *et al.* 2006, Goodwin *et al.* 2006a, Goodwin *et al.* 2006b, Jeong *et al.* 2008). The use of PCR analysis involves the preparation of tissue homogenates, DNA extraction and amplification using optimised PCR assays. Advances in technology and the fact that the method of standard PCR has many drawbacks, including the requirement of gel electrophoresis analysis of results, have led to an increase in the use and application of real-time or quantitative PCR in viral disease diagnosis in fish.

Quantitative PCR

Developed in 1992 (Higuchi *et al.* 1992, Higuchi *et al.* 1993), quantitative PCR has proven to be valuable for the detection and quantification of viral pathogens in fish (Dhar *et al.* 2008) as it overcomes the limitations of standard PCR. This is seen in the ability of quantitative PCR to differentiate between high viral loads, indicative of a fish dying from an active viral infection, and low viral loads, indicative of a fish dying due to some other infection but carrying the viral infection in its latent form (Goodwin *et al.* 2006b). Additionally, quantitative PCR has the further advantage of being faster and more sensitive compared to standard PCR. In recent years quantitative PCR assays employing SYBR® Green I (a non-specific DNA-binding dye) (Bowers *et al.* 2008, Dhar *et al.* 2008) and TaqMan probes (Overturf *et al.* 2001, Gilad *et al.* 2004, Munir and Kibenge 2004, Purcell *et al.* 2006) have been developed to detect and quantify numerous viral pathogens of fish, hence its importance as a tool for determining the virus status

in fish hosts. Among the methods of quantitative PCR, TaqMan quantitative PCR is the most costly because it requires the design of fluorescently labelled oligoprobes whereas detection by SYBR® Green I does not.

Overall, PCR is expensive, technically demanding and requires specially trained personnel. In addition the efficacy of PCR for viral detection in commercial applications is at present very limited due to its highly specific nature making it susceptible to false positives if the diagnostic procedure is carried out in a virus contaminated area (Bowers *et al.* 2008). In spite of these limitations, and as primer sets for viruses become established, the application of PCR is increasingly becoming recognised as a realistic tool for routine virus screening which would improve the management of viral diseases in not only commercial hatcheries but also populations of imported ornamental fish. A review of the literature shows that the number of PCR assays described is considerably less than the number of PCR assays which have actually been optimised and validated. This highlights the need for tests to detect exotic agents such as *Megalocytivirus* and *CyHV-2* and the specific optimisation and validation of these tests for future consideration for clinical and commercial application.

Concluding remarks

As Goodwin *et al.* (2006b) highlights, due to the difficulty of growing viruses in cell culture and the fact that there are limited published PCR assays, the distribution and incidence of many fish viruses are not known. Consequently screening of ornamental fish populations is required to determine the distribution of important exotic viral pathogens such as *Megalocytivirus* and *CyHV-2* in Australia.

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