ORNAMENTAL FISH TESTING PROJECT
FINAL REPORT

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Fish for the future

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List of Abbreviations

AAHC	Aquatic Animal Health Committee
AAHL	Australian Animal Health Laboratory
ACIAR	Australian Centre for International Agricultural Research
ANZSDP	Australian and New Zealand standard diagnostic procedure
ANZSDPS	Australian and New Zealand standard diagnostic procedures
AQIS	Australian Quarantine and Inspection Service
AQUAVETPLAN	Australian aquatic veterinary emergency plan
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAFF	Department of Agriculture Fisheries and Forestry, Canberra
EUS	Epizootic ulcerative syndrome
FRDC	Fisheries Research and Development Corporation
GFHNV	Goldfish haematopoietic necrosis virus
ICON-AQIS	Australian Quarantine and Inspection Service's import conditions database
NAAH-TWG	National Aquatic Animal Health Technical Working Group
NATA	National Association of Testing Authorities
NAHLSS	National animal health laboratory services strategy
OCVO	Office of the Chief Veterinary Officer
OIE	World Organisation for Animal Health (in French: Office International des Épizooties)
PCR	Polymerase chain reaction (a method of copying DNA)
SCAHLS	Sub-Committee on Animal Health Laboratory Standards
SDP	Standard diagnostic procedure
SPS	World Trade Organization Agreement on the application of sanitary and phytosanitary measures
TEO	Technical executive officer

Executive Summary

Biosecurity Australia commissioned a program of testing of goldfish, cichlids, gouramis and livebearers in quarantine. These species were identified as high risk in the 1999 Import Risk Analysis on Live Ornamental Finfish. In the program reported on here, these species were targeted for diagnostic testing when more than 25% of fish in a quarantine tank died during the quarantine period. Diagnostic testing usually involved post mortem, histology and bacteriology with provision for further confirmatory diagnosis as required.

Participating diagnostic laboratories in Queensland, New South Wales, South Australia and Victoria were provided with details of basic disease testing procedures and requirements to ensure a consistent approach across all states. Procedures were agreed with AQIS and established nationally to supply targeted fish to the testing laboratories.

One hundred cases were investigated from the five states. Victoria had the most submissions (43), followed by Queensland with 29 and Western Australia with 15.

A bacterial cause was diagnosed in 26 cases and in 13 of these cases the bacterium was *Aeromonas hydrophila* or *Aeromonas* sp. 41 diagnoses of protistan and metazoan parasites were made and of these the greatest number were Monogenea (14 cases). There were eight reports of fungal involvement including seven cases of epizootic ulcerative syndrome (EUS). Viral aetiologies were listed in seven submissions. These included four cases of iridovirus in cichlids and one of haematopoietic necrosis virus in goldfish (GFHNV). Stress was reported as a contributing factor in at least 11 submissions.

The large number of diagnoses of EUS, all in gouramis, and the four cases of iridovirus in cichlids is of concern. Surprisingly iridovirus was not diagnosed in gouramis during the survey despite most previous diagnoses of this virus in ornamental fish in Australia being in gouramis.

Background

The disease testing program which is the basis of this report was commissioned by Biosecurity Australia in order to gain a better understanding of the health status of imported ornamental finfish, the type and extent of disease incidents and associated biosecurity risks. The project focussed on the "high risk" species as identified in the 1999 import risk analysis of ornamental finfish; namely, goldfish, live-bearers, gouramis and cichlids.

Ornamental fish form what is probably the largest pet industry in Australia in terms of numbers of animals yet there is no firm estimate of the value of the trade within Australia. The value of the trade (including food, accessories, tanks) was estimated to be A$135-150 million per annum and in Australia there are estimated to be about 800 pet fish shops employing around 5,000 people (Kahn *et al.*1999). Ornamental fish imports were estimated to be worth $4.7 million in 2004-05 (ABARE 2006). The Pet Industry Association of Australia (PIAA) estimates that imported ornamental fish make up 40% of fish supplied to retailers (BRS 2005).

This makes the ornamental fish industry one of the larger aquaculture industries in Australia, yet one which is largely hidden from view. The ornamental industry in Australia is part of a world-wide trade in fish and fish products involving over 100 countries (Cheong 1996) yet there is very little information available on the extent of imports (and exports) or the disease risks they pose. It should be noted that export of Australian native fauna, specifically crustaceans, has resulted in the introduction of Australian parasites and diseases to other countries (Avenant-Oldewage 1993), and it is certain that the same pattern will be true for live fish exports.

There is also good evidence that the importation of live fish for the aquarium trade has resulted in the introduction into Australia of parasites and disease (Langdon 1990; Dove *et al.* 1997). In 1999 the Australian Quarantine and Inspection Service (AQIS) completed and published an import risk analysis on live ornamental finfish. Following this risk analysis, risk management measures were implemented with the aim of reducing the risk of a number of specific disease agents entering Australia in ornamental fish. However, data on the efficiency of the quarantine system since 1999 is lacking, though Evans & Lester (2001) did provide some data on parasites of ornamental fish entering Australia through Queensland.

Methods

The Australian Quarantine and Inspection Service (AQIS) monitors imported ornamental finfish during a one to three week post-arrival quarantine detention period. During this project when AQIS officers suspected the presence of a disease agent of quarantine concern or when tank mortalities were consistent or increasing in quarantine and exceeded 25% within individual tanks, then samples from the shipment were sent for diagnostic testing to the local State government approved laboratory (Appendix 1). Testing procedures were coordinated by Western Australia, starting in May 2004 and finishing in November 2006. The timeframe for submissions was extended in consultation with Biosecurity Australia because fewer samples than expected were received and there was a desire to confirm whether there were any real seasonal trends in submissions.

Participating diagnostic laboratories were provided with basic testing methodology with respect to gross examination of fish, necropsy procedures and sampling techniques (Appendix 2). Each laboratory used its standard techniques for procedures such as fixation, embedding, cutting and staining for histology and for bacteriology. Additional testing included transmission electron microscopy, tissue culture or submission of samples to bacteriologists or parasitologists for a second opinion, as appropriate. This additional testing was at the discretion of each state laboratory but pathologists sometimes sought guidance from the co-ordinating laboratory in Western Australia. Samples from one submission were sent to the Australian Animal Health Laboratory (AAHL) for virus isolation by tissue culture. Molecular techniques such as polymerase chain reaction were an option for additional testing, but were not used on any submissions in this project probably because suitable samples and test methods were not available. On completion of the cases a copy of the laboratory report was sent to the Fish Health Unit in Western Australia.

Laboratories were reimbursed the costs of testing and transport of samples to laboratories, on receipt of tax invoices, either on a quarterly basis or per case.

Cases were entered in a database (Microsoft Excel) containing all the information gathered during the project. A sample database worksheet is shown in Appendix 3. Data from Western Australian cases between 1998 and 2003 (inclusive) are also provided (see Appendices 4 and 5). Progress reports and financial information was exchanged with Biosecurity Australia by email on a monthly basis. Initially it was intended that quarterly reports would be provided, however, these were discontinued after the first report because submissions were erratic and the total number of submissions was much less than anticipated.

Regular contact was kept with laboratories in each state and with AQIS personnel in order to ensure that lack of submissions was not due to lack of interest. The final report on the project includes details of cases, an assessment of the health status of imported fish and identifies areas of concern such as fish species, sources or disease agents of significance. The report includes details of each case by jurisdiction and by species together with an analysis of any patterns and trends in the data.

Results

A total of 100 species submissions were received that match the criteria of fish to be surveyed. These included 20 submissions of livebearers, 31 submissions of cichlids, 28 gourami submissions and 21 goldfish (*Carassius auratus*) submissions, see Figure 1.

Within each major group of fish there were a number of species or varieties submitted. Livebearers included swordtails and platys (*Xiphophorus* spp.) and guppies (*Poecilia reticulata*). There were a diversity of cichlids submitted including discus (*Symphysodon* spp.), angel fish (*Pterophyllum scalare*), oscars (*Astronotus oscellatus*) and African rift valley cichlids including Kribensis (*Pelvicachromus pulcher*), Auratus (*Melanochromis auratus*), golden remirezi (*Microgeophagus ramirezi*) and blue rams (*Apistogramma raminez*). Gouramis included kissing gourami (*Helostoma temmincki*), dwarf or blue gourami (*Colisa lalia*), honey sunset gourami (*Colisa chuna*), pearl gourami (*Trichogaster leeri*), moonlight gourami (*Trichogaster microlepis)* and opaline, golden or three spot gourami (*Trichogaster trichopterus*).

The country of origin of the shipment was often not disclosed on paperwork that accompanied the samples, however, where the country of origin was disclosed it included Singapore, Hong Kong, Indonesia, Thailand and Malaysia. Businesses in Singapore and perhaps other places often act as receival and distribution outlets for fish from all over the globe. Thus, the country of origin may not indicate the true country of origin of the fish in a consignment because fish from various sources may have been mixed at these businesses and held for the 14 days stipulated by AQIS for live freshwater fish (other than Salmonidae) on its Import Conditions Database (ICON-AQIS).

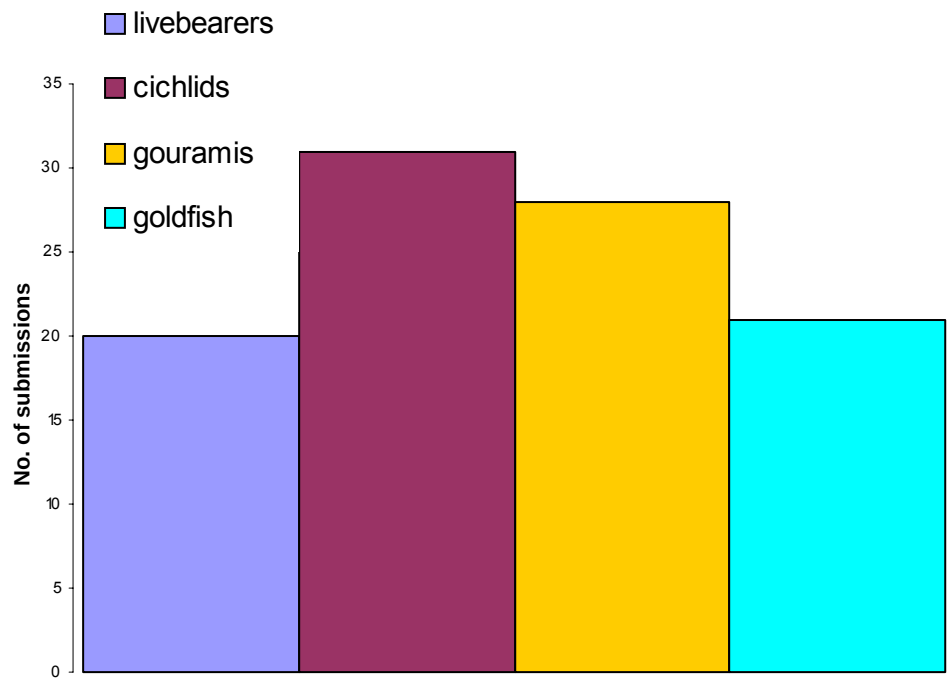
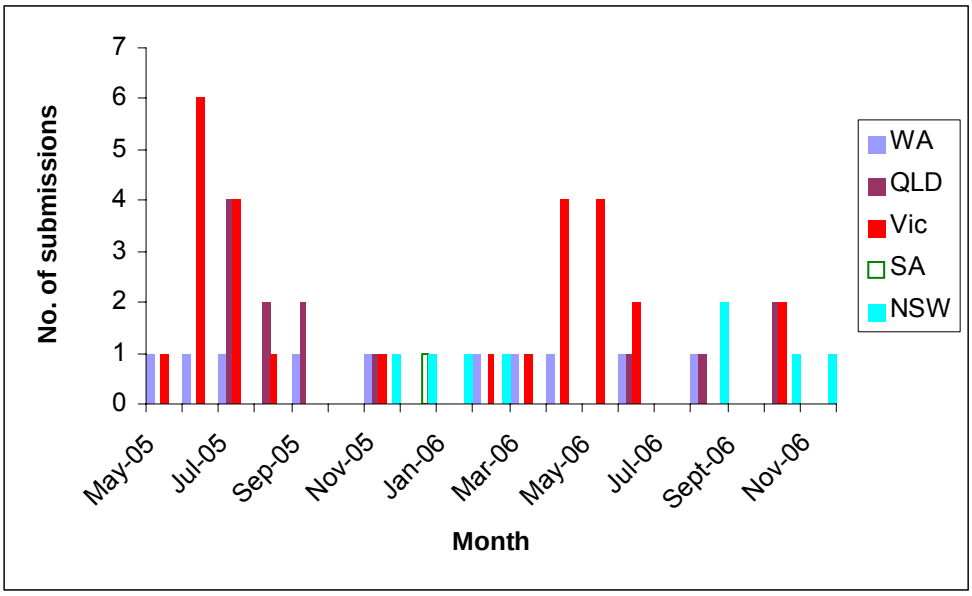
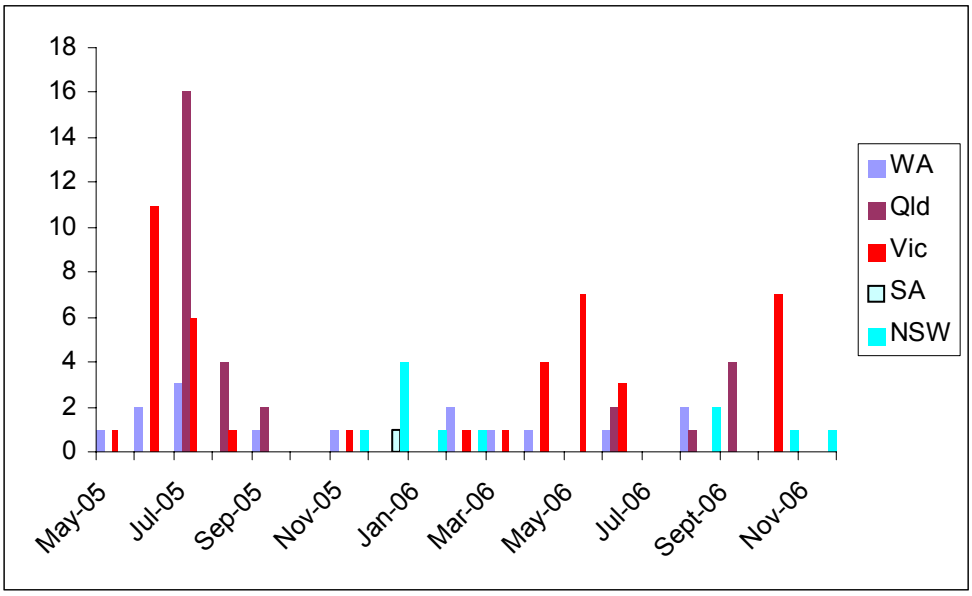


Figure 1. Total number of submissions shown by major groupings of fish species.





B

Figure 2. Submissions received in each state over the period of the project. (A) shows the number of days on which samples were received at laboratories and (B) shows the number of cases by species recorded by the laboratories. The differences can be explained by several species or varieties of fish being received at one time.

There was no apparent pattern of submissions (Figure 2). Victoria had the largest number of submissions (samples were received on 27 days) followed by Queensland (13), Western Australia (10), New South Wales (8) and South Australia (1). There are two peaks in submissions. The first was at the start of the project (June and July 2005) and the second was in April to June 2006.

A large number of diagnoses were made (Tables 1, 2 and Figure 3). In some cases only morphological diagnoses were possible (e.g. skeletal muscle damage or skin damage were noted but no aetiological diagnosis was made). In a number of cases more than one parasite or pathogen was identified and all of these have been listed in Tables 1 and 2. In some instances the samples were sent to specialists for further testing or identification but usually a less specific identification of parasites based on morphology in histology sections was provided. There were some noticeable differences in quality between the reports from various

laboratories and pathologists. In some instances the ability to identify common pathogens improved as the survey progressed and at other times a second opinion was sought and this improved the diagnosis.

Fish pathologists at the co-ordinating laboratory interpreted some results in an attempt to categorise them. This was done when diagnoses included statements about stress and/or water quality issues and also when the histological description of lesions fitted a syndrome or disease such as *Cryptobia* sp. in cichlids.

Infection with bacteria, most commonly *Aeromonas hydrophila* or *Aeromonas* sp., was the most frequently reported diagnosis but there were no reports of the non motile *Aeromonas salmonicida* or its atypical forms that cause furunculosis in salmonids, goldfish ulcer disease and carp erythrodermatitis. There were seven diagnoses of epizootic ulcerative syndrome (EUS) in gouramis submitted in three states and the four cases of iridovirus in cichlids, including two in red albino oscars, one from Western Australia and one from Victoria. Interestingly there was only one diagnosis of haematopoietic necrosis virus of goldfish but a diversity of metazoan and protistan parasites were seen in this group of fish. Goldfish were infected with unidentified species of microsporidia, *Goussia* sp. and *Myxobolus* sp. that are not commonly listed as pathogens of this species. There were no reports of spring viraemia of carp.

Diagnosis	WA	Qld	Vic	SA	NSW
No diagnosis			11		3
Bacterial- unspecified	3				
Pseudomonas sp.					1
Streptococcus sp.			1		
Mycobacteriosis	1	2	1		1
Plesiomonas shigelloides			1		
Aeromonas spp.	1		12		
Epitheliocystis		1	1		
Water quality/stress	2	7	1	1	
Parasitic- unspecified			5		
Monogenea	1	3	10		
Chilodonella sp.					1
Ichthyophthirius multifilis	1	1	3		1
Cryptobia sp.	1		1		
Goussia carpelli					1
Microsporidia					1
Myxozoa		1			1
Coccidiosis		1			
Tetrahymena		2			
Trichodina sp.			2		
Argulus sp.					1
Centrocestus formosanus		1			
Metazoa- other		1			

Diagnosis	WA	Qld	Vic	SA	NSW
Viral					
Iridovirus	1	1	2		
lymphocystis	1	1			
Herpesvirus			1		
Oomycete/fungi					
EUS	1		3		1
Saprolegnia-like					1
Miscellaneous pathology					
skeletal muscle damage	2	3			
skin damage	1				
kidney damage		4			1
retina vacuolation			1		
splenitis			1		
granulomas-unspecified origin	1		1		
TOTAL	23	33	60	1	14

Table 1. Diagnoses made by each state.

Diagnosis	***Carassius auratus***	***Helostoma temmincki***	***Colisa lalia***	***Trichogaster* sp.**	***Xiphophorus* spp.**	***Poecilia* sp.**	***Symphysodon sp.***	***Astronotus oscellatus***	***Pterophylium* sp.**	***Microgeophagus* sp.**	***Melanochromis auratus* (Auratus)**	***Apistogramma sp.***	**Total**
No diagnosis	7					4	2	1		1			15
Bacterial- unspecified	1	1						1					3
Pseudomonas sp.	1												1
Streptococcus sp.									1				1
Mycobacteriosi	1		2		1							1	5
Plesiomonas			1										1
Aeromonas sp.	3				2	3	2	2	1				13
Epitheliocystis								2					2
Water quality/stress	2		1		1	2		1	2		1	1	11
Parasitic- unspecified	3						1		1				5
Monogenea	3		1	2	2	1	1	4					14
Chilodonella									1				1
Ichthyophthirius multifilis	3	1		1	1								6
Cryptobia sp.							1		1				2
Goussia sp.	1												1
microsporidia	1												1
Myxozoa	1					1							2
Coccidiosis				1									1
Tetrahymena sp.						2							2
Trichodina sp.				1		1							2
Argulus sp.	1												1
Metazoa-other	1				1	1							3

Diagnosis	***Carassius auratus***	***Helostoma temmincki***	***Colisa* sp.**	***Trichogaster* sp.**	***Xiphophorus* spp.**	***Poecilia* sp.**	***Symphysodon* sp.**	***Astronotus oscellatus***	***Pterophylium* sp.**	***Pelvicachromis pulcher* (Kribensis)**	***Melanochromis auratus* (Auratus)**	***Apistogramma* sp.**	**Total**
Viral													
Iridovirus								2		1		1	4
lymphocystis	1	1											2
Herpesvirus	1												1
Oomycete/ Fungi													
EUS		3	4										7
Saprolegnia-like			1										1
Miscellaneous pathology													
skeletal muscle damage	3		1	1		1							6
skin damage						1							1
kidney damage	1		3				1						5
retina vacuolation					1								1
Splenitis									1				1
granulomas-unspecified origin	1						1						2

Table 2. Diagnoses by fish species

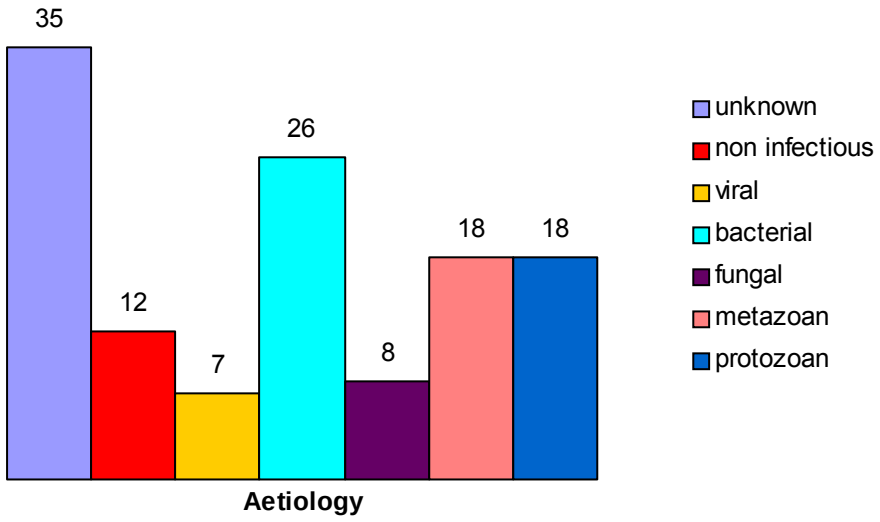


Figure 3. Diagnoses grouped into major causes of disease.
There was more than one cause listed for some cases.

Discussion

Diseases of concern

Iridovirus consistent with a Megalocytivirus was diagnosed in four submissions of cichlids including red Oscars in two states. In one of the submissions of oscars there were several other pathogens noted and it is conceivable that immunocompromised fish with chronic iridoviral infection are more likely to become heavily infected with other pathogens and opportunists. Published reports of Megalocytivirus in ornamental fish usually report the disease in gourami (Go *et al.* 2006), however, the disease was not diagnosed in gouramis in this survey despite several previous cases having been received from fish in AQIS quarantine premises (Appendix 4). This virus was identified as a high priority risk in gouramis (Kahn *et al.* 1999). The severity of disease in the affected cichlids and the number of cases suggest that the two week quarantine period for gouramis and cichlids is warranted. The import risk assessment for these viruses should be reviewed in light of the cases seen during this survey and the occurrence of a similar virus in Murray cod which was attributed to infection with a virus originating in imported fish (Go *et al.* 2006). Implementing similar risk management practices to those for imported goldfish (ICON-AQIS) may be appropriate under the circumstances.

Epizootic ulcerative syndrome (EUS) was reported in seven cases of gouramis from a number of states (Table 1 and 2). This disease is listed as a disease of significance by the World Organisation for Animal Health (OIE) and is reportable in Australia to the Department of Agriculture, Forestry and Fisheries. EUS is endemic to Australia and, as such, was not considered in the risk assessment of the 1999 Import Risk Analysis (Kahn *et al.* 1999). Gourami in this survey appeared to have longstanding, subclinical infections and it is easy to imagine the dissemination of this organism by imported fish (David Alderman, personal communication, 2002; Lilley *et al.* 2003). There are no restrictions on

the movement of live fish within Australia in relation to EUS and there is no published information about strain differences in the causative agent *Aphanomyces invadans.* However, it is likely that there are strain variations (Lilley *et al*. 2003; Heather McLetchie, pers. comm. December 2006, FRDC Projects 2001/621 and 2004/091). Species vary in their susceptibility to EUS and in their immune response following infection (Catap & Munday 2002). Catap found that three spot gouramis were less susceptible to developing severe clinical disease than other species that she studied. It is therefore likely that asymptomatic carrier fish are provided with health certificates in the country of origin because Australia's current import requirements only require there to be "no clinical signs of infectious disease or pests" prior to export. There is no requirement for histological or microbiological examination of gouramis. Goldfish are the only fish currently required to have a surveillance and monitoring program for specific diseases including spring viraemia or carp and *Aeromonas salmonicida* (ICON-AQIS). As with most diseases, the stresses of transport, water quality aberrations and assimilation to a new environment before and after arrival in Australia would be sufficient to precipitate overt disease and mortality from EUS. It is also possible that these fish may have concurrent infection with opportunistic pathogens such as *Aeromonas hydrophila*. Therefore, because of the number of cases seen in this survey, revisiting the risk assessment for this disease seems warranted.

The presence of some unusual parasites such as the coccidian *Goussia carpelli* in the intestines of goldfish; *Myxobolus* sp. in goldfish; *Sphaerospora* sp. in guppies; lesions consistent with *Cryptobia iubilans* in cichlids; *Centrocestur formosanus* in goldfish and unidentified microsporidia in a variety of fish, is of interest. The risks associated with these parasites are unknown and thus have generally been interpreted as insufficient to pose a significant biosecurity risk, notwithstanding that the viviparous freshwater monogenean parasite *Gyrodactylus salaris* is of sufficient concern to be OIE listed. The import risk analysis (Kahn *et al.* 1999) was based on meeting the criteria of the SPS agreement and there was no attempt to assign risk to potential pathogens of uncertain risk status that may be entering Australia with ornamental fish. *Cryptobia iubilans* and other species of *Cryptobia* were not mentioned by Kahn *et al.* (1999) but there is evidence that *Cryptobia* species are now common parasites in Australian cichlids and are often diagnosed histologically in subclinically infected fish or in fish that have recovered from infection and have residual granulomas. Evans & Lester (2001) reported 66% of *Gyrinocheilus aymonieri* (sucking catfish) examined by them were carrying *Cryptobia* sp. (probably an undescribed species). What species of *Cryptobia* have established in Australia is unknown. *Cryptobia iubilans* causes serious disease in young or stressed fish (Yanong *et al.* 2004) and is one parasite that would warrant further attention during any review of the import risk analysis of ornamental fish. A parasite resembling the digenean *Centrocestur formosanus* was found in goldfish from a retail outlet in Western Australia in 2006 and has been reported in Turkey in imported goldfish (Yildiz 2005). The significance of the spread of this and other parasites will not be known until there is evidence of their establishment or non establishment in other regions or species.

A large number of bacteria were isolated from the fish but none of these are considered to be primary pathogens and none cause significant or reportable diseases. They are more likely to be opportunistic infections following the stresses of transport and less than optimal water quality.

Subclinical disease may go undetected and this is a risk that warrants further investigation in risk assessment. One disease that was identified as a biosecurity risk and for which

disease management strategies were implemented after the 1999 import risk analysis was goldfish haematopoietic necrosis virus (GFHNV). Goldfish haematopoietic necrosis virus appears to have entered Australia in goldfish in the early 2000s since several laboratories in Australia diagnosed the disease at around the same time (Stephens 2002). This disease is caused by a Herpes-like virus, and fish appear to be carriers without showing clinical signs. When carrier fish are stressed they can develop clinical or subclinical disease and produce virus particles which can enter the environment and infect other goldfish. Another feature of this disease is that it targets the haematopoietic tissue that is largely responsible for the determining the efficiency of the immune system of fish. To illustrate this point, in 2004 the Western Australian Department of Fisheries received goldfish from an ornamental fish retailer for diagnosis. The fish had been imported from Malaysia seven weeks earlier and had a steady slow mortality throughout the seven week period despite the application of several anti-parasitic and antibiotic treatments. At the time of submission 80% of the fish had died. Histologically lesions in the haematopoietic tissue and gill were consistent with goldfish haematopoietic necrosis virus, but there were also dactylogyrid-like parasites and bacteria similar to *Flavobacteria* sp. in the gills and *Aeromonas hydrophila* was cultured from the blood of one fish. It was likely that the fish were dying from secondary infections resulting from immunosuppression caused by either subclinical goldfish haematopoietic necrosis or from an outbreak of clinical disease that had occurred prior to importation. It is possible that fish with this disease entered Australia as carriers or were subclinically infected or not suffering mortalities that were high enough to trigger action by AQIS inspectors.

Commonly used diagnostic techniques such as histology are unlikely to detect carriers of this and most other viral and bacterial disease and for this reason screening tests that can detect carriers need to be developed for a number of diseases if health certification and quarantine (both pre- and post-) are to be effective. GFHNV illustrates some of the deficiencies in the current AQIS risk management policy. Goldfish are required to have additional certification based on inspections by the Competent Authority in the country of origin every six month to ensure that there are no clinical signs of this disease. How "clinical signs" of disease are determined is not specified. It may be merely and visual inspection fish from the side of a pond in which case the disease is unlikely to be detected because diseased fish often display no gross external lesions. Histological examination of sick fish during clinical outbreaks of disease or a demonstrated absence of the infectious agent by regular surveillance using molecular techniques such as the one published by Goodwin *et al.* (2006) is needed if health certifications of freedom from infection are to have validity.

Additionally goldfish must be treated with an effective parasiticide within 7 days of export to Australia to manage infestations of the gill parasites *Dactylogyrus vastator* and *Dactylogyrus extensus*. Trichlophon, formaldehyde and sodium chloride are suggested as suitable treatments. Whilst these treatments will certainly remove some of these parasites, and also other parasites, their efficacy is likely to be extremely variable. These parasites are very small, infested fish show few clinical signs except increased respiratory effort during heavy infestations, are difficult to remove and eradicate from a population of fish (Thoney & Hargis 1991). They can produce a large number of eggs and populations can build up rapidly under suitable conditions, especially when fish are stressed. During the survey 5 submissions of goldfish reported as an incidental finding that dactylogyrid-like parasites were present on the gills. This finding is evidence that *D. vastator* and *D. extensus* could be entering Australia undetected.

A suggested course of action to address some of these concerns with the health of goldfish would be to implement a more detailed study of the disease status of goldfish in both imported fish in quarantine and in populations already in Australia. This should include molecular testing for GFHNV and morphologic identification to species of Monogenea found on gills.

Pattern of submissions

Logistically some issues arose during the survey. In some states there was confusion over whether fish presented for diagnosis were species that were targeted as part of the ornamental fish survey. This occurred most often in Western Australia where an ongoing diagnostic service is offered to AQIS for imported ornamental fish. As a result, some species were initially included that did not satisfy the selection criteria and these results were removed at a later date.

Analysis of trends is difficult because submissions showed a pattern but there were not enough submissions to assess whether this pattern was a true indicator of the level of health of imported ornamental fish. Perhaps the trend is a reflection of the number of imports received into each state, the species that were imported at particular times or a seasonal pattern in health of imports. For example, why were most of Queensland's submissions during the first two months of the project and why was there only one submission from South Australia? AQIS could use its records to clarify some of these possibilities. Alternatively there may have been certain AQIS officers who submitted more samples because they were personally more committed to the project.

Tank records sometimes showed information on other tanks at the same quarantine facility and it was not uncommon to see that mortalities of target species in other tanks had exceeded the 25% needed to trigger submission as part of the present survey but no fish had been submitted. There were no comments on the sheets to explain why these fish had not been submitted. AQIS officer's sometimes explained to us that all of the fish had died when they visited the premises. At other times they thought that management problems such as aeration failures had caused the high mortality but the records did not note such events. Such events should be recorded on tank records and more adequately investigated to rule out the possibility of disease contributing to the problem.

Quality of diagnostic reports

It was clear from the comments on the diagnostic reports received that a number of the pathologists had limited experience or training in fish pathology. For example, supporting a diagnosis of "mild parasitic branchitis":

When checking the gills for ectoparasites an irregular pattern of melanin distribution along the filaments was noted. In addition, instead of the filaments being thin and 'filamentous', these were quite thick and appeared to be full of refractile 'globules'.

The appearance of these gill filaments seemed 'unusual', but it is a species of fish I have not seen before and am unsure whether this is 'normal' or not.

At times it was difficult to interpret findings. For example, a histological finding of "Moderate infestation of the gills with flukes with significant numbers of encysted trematodes" was followed in the comments section by "Flukes are small worm-like monogenean trematodes measuring up to 2mm in length……Gill flukes…..". It is unclear whether the parasites noted in the gills histologically were Digenea that can correctly be described as flukes or trematodes, have a lifecycle involving more than one host species

and can have encysted stages in fish tissues or whether the parasites were Monogenea such as *Dactylogyrus* sp. These gill parasites are often seen in ornamental fish and are colloquially referred to as "flukes" but this term can cause confusion when used by fish health professionals.

A case reporting findings in discus noted chronic granulomas in the spleen, skin and stomach and possible fungal hyphae in the spleen. There was no reference to the use of special stains or other techniques to confirm a diagnosis and it remains unclear whether the granulomas were the result of Mycobacteria, fungi or infection with *Cryptobia* sp. (a common cause of granulomas in the stomach wall of cichlids).

Another report described hepatic lipidosis at length without being able to report any other significant findings. Fatty liver is common in fish reared in aquaculture and is generally not noted as a significant finding unless other aspects of the history or analysis suggest that it may be contributing to ill health.

It was apparent that pathologists in laboratories without a designated specialist fish pathologist had access to photographs or diagrams of fish parasites and a good understanding of mammalian pathology. They extrapolated this knowledge to fish, however, there were limitations to this process because the significance of lesions, such as gill lesions, is difficult to assess without experience or training. One method of increasing the effectiveness of diagnoses would be to send histology slides or photographs of parasites seen in wet preparations to more experienced peers for comment.

This issue is of relevance to Biosecurity Australia in that diseases entering Australia may be misdiagnosed; but it is highly probable that overseas veterinary pathologists may also be struggling to interpret aquatic pathologies. The results of this survey suggest that Australia is not well placed to withstand scrutiny of routine aquatic animal veterinary diagnostic capability by overseas auditors.

Inconclusive fish origin

It would be interesting to traceback the red albino oscars with iridovirus and the gouramis with lesions typical of EUS but under the current AQIS import conditions database (ICON-AQIS) there is no requirement to trace these species any further than the premises immediately prior to export to Australia. It would be interesting to know whether these fish came from the same farm or whether there is a much larger problem with the majority of fish of certain species being infected globally. Since these problems were readily detected by histology in Australian laboratories, the basis on which the health certificates or additional health certificates are provided needs to be re-assessed in light of a review of the risk analysis for these disease agents.

Comparison of survey results and other Western Australian ornamental fish submissions

Appendices 4 and 5 compare the diagnoses from ornamental fish in quarantine with those from ornamental finfish submitted from other sources such as hobbyists, aquarium shops, aquaculturists and aquarium maintenance businesses in Western Australia prior to the present survey. There were three cases of EUS (all in gouramis), one of GFHNV, six of cryptobiosis and four of iridovirus (all in gouramis) from fish in quarantine but one, seven, six and nil respectively from fish not in quarantine. Comparison of this historical data with results from the present survey present some interesting findings that are worthy of further consideration. Diagnosis of the latter three diseases can be difficult and requires

some previous fish diagnostic experience and/or electron microscopy. Goldfish recovering from goldfish haematopoietic necrosis virus are often infected with other agents because of their immunocompromised state and this can compound the difficulties. *Cryptobia* organisms are difficult to see histologically (hence the name, from the same root as "cryptic") but produce characteristic granulomas, disease signs and clinical history. Iridovirus inclusions can be mistaken for amoebae.

It would seem that some diseases including GFHNV and cryptobiosis of cichlids are now established in ornamental fish in Australia. The relatively large numbers of cases of GFHNV seen by the Fish Health Unit in Western Australia between 2002 and 2005 may reflect outbreaks of disease when the virus entered previously naïve populations in Australia. Cryptobiosis is usually diagnosed in cichlids in Western Australia after some weeks of ill thrift following a change of ownership or as an incidental finding. Many cases of "Malawi bloat" or "bloat" in cichlids have histological lesions typical of cryptobiosis as well as infection with *Spironucleus* sp. Occasionally cryptobiosis is seen in juvenile fish in Western Australia. In the US it was reported in juvenile discus (Yanong *et al.* 2004) and there are other less comprehensive reports of the disease in cichlids from other parts of the world. This suggests that the relatively short time of holding in quarantine is more likely to result in ill thrift than mortality from this disease. Both cryptobiosis and goldfish haematopoietic virus now seem more likely to be diagnosed in fish outside AQIS quarantine facilities In Australia. In some cases the fish were previously in quarantine but were presented for diagnosis after failure to thrive in the days or weeks after leaving quarantine.

A submission of a large number of species from a retail outlet with an AQIS import licence during this survey found a number of unusual pathogens. These included Digenea, possibly *Centrocestus formosanus*, in goldfish gills. Similar lesions were also found in a submission from Queensland a few weeks later. Digenea have at least one intermediate host but there is the possibility that a suitable intermediate host would be present in Australia. There were also Dactylogyrid-like parasites in the gills of two varieties of goldfish and protozoan-like cells in the brain stem and spinal cord.

Decision making on fate of fish in quarantine

When laboratories receive fish from AQIS because of concern about high mortalities during the quarantine period there has been confusion and concern over who should make decisions about the fate of the fish, and their legal power to do so. Pathologists in some laboratories make recommendations to AQIS officers that fish should be destroyed when notifiable or other infectious diseases of concern such as EUS or iridovirus are diagnosed. Other pathologists are justifiably reluctant to provide such advice. The legal ramifications of destroying fish that are not affected by a notifiable disease need to be considered and some guidelines should be presented to diagnostic laboratories. Fortunately there has not been a major objection to the destruction of fish to date, perhaps because almost all of the fish in the consignment have died from the disease before the diagnosis is made.

Time delay between sample taking and diagnosis

It is reasonable to expect that provisional diagnoses will take up to one week and definitive diagnoses may take a considerable time longer or often may not be possible at all. Because most species have a mandatory quarantine period of only 1 or 2 weeks the diagnostic process will often not be completed by the end of the quarantine period. Perhaps this one reason why importers may be reluctant to provide samples for diagnosis and may prefer to destroy all fish in a tank.

Variation in diagnostic techniques

Diagnoses made during this survey varied considerably in their specificity. In most cases further testing could be have done to improve the accuracy of the diagnosis. For example all five diagnoses of EUS were made on the basis of histology with granulomatous myositis and large fungal hyphae in histological section. Culture or PCR were not used to confirm the diagnosis. Similarly with diagnoses of iridovirus infections the appearance of typical basophilic inclusions in histological sections, sometimes backed up by the finding of iridovirus-like particles in transmission electron microscopy, was the basis of diagnosis. PCR was not performed.

Similarly the identity of a few metazoan parasites were confirmed morphologically by specialists with an interest in the specific group of parasites, but more commonly a diagnosis was made from the appearance of parasites in histological sections and their similarity to those in the same fish species in published articles.

If Biosecurity Australia is to make any significant improvement in the accuracy of health certificates of imported ornamental fish it is imperative that the method of diagnosis is stated. Additionally Australia needs to supply details of acceptable test methods, as it has done for infectious viraemia of carp. As new and improved diagnostic methods become available these need to be included as the required test methods. Examples of this deficiency are the goldfish haematopoietic necrosis virus and *Dactylogyrus* in goldfish. There are currently no diagnostic methods stated and it is unlikely whether "gill flukes" found on goldfish have been identified by a specialist in the morphology of Monogenea. Likewise routine testing of goldfish histologically is unlikely to find evidence of goldfish haematopoietic necrosis virus. The recently published PCR for detection of this virus (Goodwin *et al.* 2006) might provide the ability to identify populations that are infected with or free of the virus. Similarly, as diagnostic tools are developed that should be validated and assessed for their potential use in screening imported fish.

Areas of concern

1. The Import Risk Analysis for gouramis and cichlids, especially in relation to EUS and Megalocytovirus (iridovirus) would appear to be in need of review in the light of recent publications and the results of this project.

2. In several laboratories there were no pathologists with specialised training in diagnosis of aquatic diseases and pathogens. This means that diseases entering Australia may be misdiagnosed, but it is highly probable that overseas veterinary pathologists are also struggling to interpret aquatic pathologies. Australia is not well placed to withstand scrutiny of routine aquatic veterinary diagnostic capability by overseas auditors.

3. At present, diagnostic techniques, sampling protocols and treatment methods are not specified for diseases for which management strategies and health certificates are required. Under the current Import Conditions (ICON) database these includes goldfish haematopoietic necrosis virus, *Dactylogyrus extensus*, *D. vastator* and *Aeromonas salmonicida* (atypical strain). The importation of asymptomatic carriers of disease will continue to be a problem until robust, rapid diagnostic techniques preferably based on PCR (for viruses) are developed and used as a basis of health certification.

4. Kahn *at al.* 1999, p.119. recommended that the Competent Authority, in countries producing large numbers of goldfish that are imported into Australia, should be audited. This audit process may need to be reviewed given that GFHNV is now in Australia and that fish are entering with dactylogyrid –like parasites.

5. Prior to this survey it would appear that testing of imported fish at the point of entry was rarely undertaken in Australia. Increase testing of batches of ornamental fish that suffer high mortality in quarantine and the provision of instructions to officers and laboratories on how to manage these batches after diagnosis would be useful.

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Appendix 1. Work Instruction to AQIS Officers

BIOSECURITY AUSTRALIA ORNAMENTAL FISH DIAGNOSTIC TESTING PROJECT: WORK INSTRUCTIONS FOR AQIS FISH INSPECTORS

Biosecurity Australia is conducting a disease diagnostic testing project aimed at obtaining better information about the health status of imported ornamental fish for diseases of quarantine concern. The objective of the project is to provide Biosecurity Australia with a better understanding of the type and extent of disease incidents to improve biosecurity risk assessments and develop better instructions for AQIS inspectors on how to deal with disease problems in ornamental fish quarantine premises.

The project will be managed and coordinated by an external consultant (the Western Australian Department of Fisheries) ─ the Project Coordinator. The project will focus on the "high risk" species as identified in the 1999 import risk analysis (IRA) of ornamental finfish, i.e.

(1) goldfish – all varieties of *Carassius auratus*;
(2) cichlids – all members of the family Cichlidae;
(3) gouramis – all members of the subfamily Trichogastrinae of the family Osphronemidae;
(4) livebearers – all members of the family Poeciliidae. The project will commence 30th May 2005 and is planned to run for one year.

During the project, should an AQIS fish inspector suspect the potential presence of a disease agent of quarantine concern (guidelines are below), they should direct samples of fish from the affected tank for diagnostic testing at the local State government laboratory (contact details are below).

Before fish are sampled, the importer's consent must be obtained using the attached 'Agreement of transfer of ownership' (Appendix 1-A).

Guidelines for determining whether a disease agent of quarantine concern is potentially present

- One or more fish from a particular shipment exhibit signs of clinical disease, eg death, external lesions, abnormal behaviour (eg flashing, gasping, inactivity etc), and;

- The clinical signs cannot be reasonably explained as being caused by a factor not associated with a communicable disease agent, eg travel stress, heat stress, water oxygen deficiency and;

- The tank records show a pattern of consistent or increasing mortalities over time (i.e. not falling off) and cumulative mortalities exceed 25% of fish in a single tank. A single shipment of fish may be housed in one or more quarantine tanks.

Sample collection/submission

1. The 'Agreement of transfer of ownership' (Appendix 1-A) must be completed and signed before collection of fish for testing. This form must be forwarded to AQIS (see contact officer details below). The form does not accompany the shipment.

2. 5-10 live affected fish should be collected for submission to the diagnostic laboratory (see Appendix 1-B).

3. Fish should be packed in a plastic bag with 1/3 water and 2/3 air, with the top of the bag tied closed. After filling with water and placing the fish inside, the air in the bag should be squeezed out and the bag filled with oxygen (or air, if oxygen is unavailable). To ensure the bag is air/water tight, twist and double-over the neck of the bags and tie using tape, cord or rubber bands.

4. Fish collected for diagnostic testing must be alive and should show clinical signs. NOTE: dead fish cannot be tested.

5. Submissions must include a copy of the tank record, contact details of the importer and the AQIS officer, to allow early feedback on test results.

6. The bags must then be packed in a watertight 6-pack foam esky, sealed and clearly labelled "Diagnostic Specimens – perishable", followed by the delivery address.

7. Fish must be sent by courier as soon as possible to the diagnostic laboratory. Overnight transport is preferable.

8. Each State diagnostic laboratory has a standard submission form for their individual lab.

9. AQIS Officers, by using the preferred courier service for each laboratory, must tick the charge receiver box on the consignment note. It is the diagnostic laboratory's responsibility to pay the courier. Cost of testing and sample shipment will be borne by the Project Coordinator.

10. The diagnostic laboratory should be advised of the imminent arrival of live fish submission.

11. Results of diagnostic tests will be provided by the Project Coordinator to the regional AQIS office with a copy to AQIS Canberra

Appendix 1- A: Agreement for the Transfer of Ownership in Imported Ornamental Finfish

Date

Parties

Name	
Short form name	**Transferor**
Notice details	

Name	Commonwealth of Australia (Commonwealth) for the purposes of this Agreement represented by and acting through the Department of Agriculture, Fisheries and Forestry
Short form name	**Transferee**
Notice details	

A: Background

A The Transferor is the beneficial owner of the diseased imported ornamental finfish ('the property').

B The Transferor has agreed to transfer ownership and the Transferee has agreed to accept the property on the terms and conditions contained in this agreement.

C The Transferee will use the property for the purpose of an aquatic surveillance program being conducted by Biosecurity Australia ('BA'), which is a part of the Transferee.

B: Agreed terms

1. **Transfer of Ownership**
   Transferor, as beneficial owner, of the property, agrees to transfer ownership of the property to the Transferee and the Transferee agrees to take ownership of the property from the Transferor and undertakes to dispose of the property at its own cost at the conclusion of the BA aquatic surveillance program.

2. **Other costs**
   The Transferee acknowledges that it is liable for all costs associated with the disposal of the property at the conclusion of the aquatic surveillance program.

3. **Warranties**
   The Transferor warrants that it is the owner of the property identified and that the property is unencumbered.

Title and Risk

3.1 Title

Title to the property delivered passes to the Transferee on the signing of this agreement.

3.2 Possession

Possession of the property and risk related to the property is given by the Transferor and taken by the Transferee at the delivery point at the time of delivery.

Governing Law and Jurisdiction

4.1 Governing Law

This agreement is governed by the law applicable in Australian Capital Territory.

4.2 Jurisdiction

Each party irrevocably and unconditionally submits to the non-exclusive jurisdiction of the Courts of the Australian Capital Territory.

EXECUTED as an agreement

Signed **by** **of** **[insert Transferor Company Name] for and on behalf of**

←	←
Signature of witness	Signature of Transferor
Name of witness (print)	Name of Transferor (print)

Signed for and on behalf of the **Commonwealth of Australia** by a duly authorised representative

Signature of witness	Signature of authorised representative
Name of witness (print)	Name of authorised representative (print)

Appendix 1-B: State Diagnostic Laboratories

New South Wales
NSW Fisheries Regional Veterinary Laboratory
Bruxner Highway Wollongbar NSW 2477

Queensland
Yeerongpilly Veterinary Laboratory
Animal Research Institute, 665 Fairfield Road Yeerongpilly Queensland 4105

South Australia
Gribbles Veterinary Pathology
33 Flemington Street Glenside SA 5605 1300 307190

Western Australia
Fish Health Laboratory
Department of Agriculture 3-Baron Hay Court South Perth WA 6151

Victoria
Primary Industries Research Victoria, Attwood Site
475 Mickleham Rd Attwood Victoria 3065
03 9217 4200 fax 9217 4199

Appendix 2: Post-mortem Procedure

FINFISH

1. Purpose and Scope

The purpose of this procedure is to provide a guide to laboratory staff conducting finfish post mortems to enable a consistent approach. This procedure applies to all finfish post mortems.

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2. Procedure

The following post mortem procedure describes a basic technique for finfish.

External examination

- The surface of the necropsy table, dissecting board and equipment are cleaned and treated with Virkon® or ethanol between cases or animals to reduce contamination of the working area.
- Pots containing buffered formalin 10% (diluted in seawater or freshwater with buffering salts) sterile bacteriology sample containers/bottles, syringes/needles and swabs expected to be used during the post mortem are labelled with the Case number prior to commencing.
- All equipment needed to perform the post mortem is placed together including forceps, scalpel, scissors and any other equipment necessary. The size of the sample will dictate other necessary equipment.
- The wearing of a plastic apron, enclosed shoes and gloves is mandatory for all staff carrying out post mortems.
- After euthanasia, place the fish on the dissecting board or in a shallow dish where necessary. Convention states that fish are drawn/photographed from the left lateral view (i.e. with the head to the left).
- A worksheet is used to record each batch of specimens and for each individual specimen when greater detail is required.
- Systematically check the outer surface, fins, eyes, nares (nostrils), oral cavity and under the operculum. Look at the gills in situ and note their colour and any discoloured areas or ragged appearance. Spread the fins and check for parasites.

- **Skin:** Scrape the skin in the direction of the scales (craniocaudal). This is best done behind the pectoral fin and on any visible lesions and examine under low power with a drop of water. Motile protistan parasites and monogenean flukes are common. Giemsa or Gram stains may be performed. NOTE: Skin scrapings can be done on live fish.
- **Gills:** Lift the operculum or remove with scissors. Larger gill samples may be removed and examined under low power in a Petri dish of water. Examine a few primary lamellae under high power. . Motile protistan parasites and monogenean flukes are common.

NOTE: A gill biopsy can be performed on a live fish over 3 cm in length. The operculum is lifted with forceps and the tips of 2-3 gill filaments snipped off with fine scissors. Haemostatis is instantaneous. Examine gill specimen in a drop of water.

- **Blood:** Sample may be taken from the caudal vein or the sinus (*ductus Cuvieri*) behind the posterior wall of the opercular cavity, or from the heart through the body wall.
- **Eyes**: Examine for parasites and exophthalmos. Eyes may be removed to check for haemorrhage, gas or oedema fluid or other abnormality behind the eyeball.
- **Nares:** Cut nares (nostrils) and examine a smear of the lining.
- **Anus:** Check for distension, reddening, ulceration or discharge.

Internal examination

- Make a cut along the ventral surface immediately anterior to the anus. Insert scissors and cut forward to the base of the pectoral fins. Cut through the pectoral girdle and then cut up the edge of the operculum to the top of the abdominal cavity and then back towards the vent (ensuring that the internal organs are not ruptured) so that the flap of the body can be removed. Refer to Figure 1

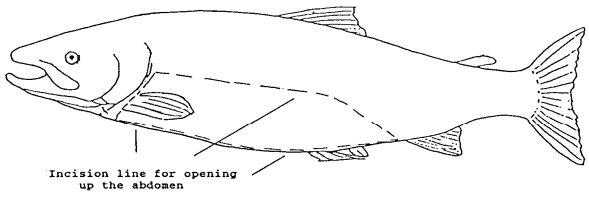

Figure 1: Representation of the incision line for internal examination.

- Check for ascites. Observe grossly the liver, spleen, heart, stomach, intestines, abdominal fat, swim bladder, peritoneum, muscles and kidney. Check abdominal organs for colour, friability, adhesions, haemorrhages, necrosis, nodules, cysts and parasites. Incisions should be made in muscle blocks to check for haemorrhage and parasites. Refer to Figure 2.

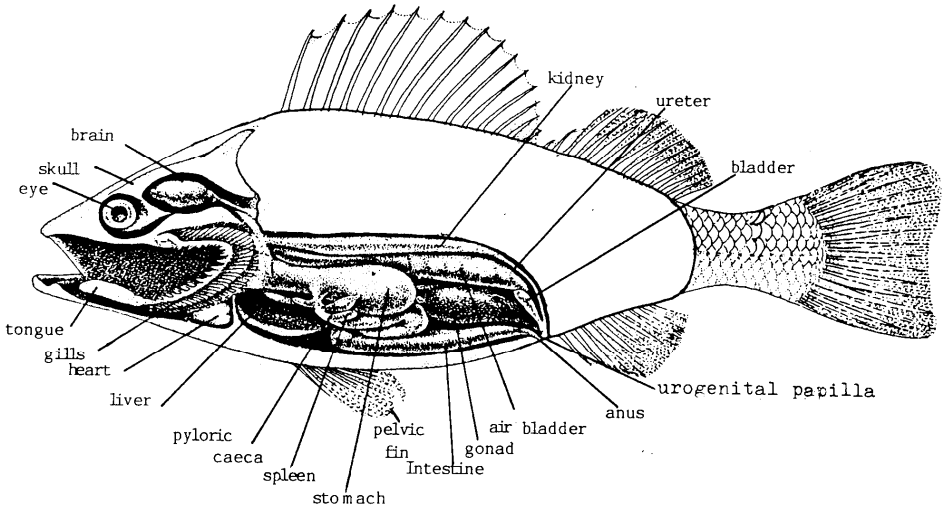

Figure 2: Internal organs of a bony fish.

- To check for endoparasites, remove the internal organs into a Petri dish, open the gastrointestinal tract along its length, check the presence or absence of food and any lesions on the walls. Scrapings should be examined for metazoan and protistan parasites by wet smear. Intestinal contents can be sieved; the size necessary will depend on the specimen. Observe the size and colour of the gall bladder. Smear the contents of the gall bladder.
- To examine the brain, cut into the cranium, posterior to the eyes with a bone-cutting instrument. The brain in finfish is small and requires careful extraction. When the cavity is exposed, lift the brain out with forceps (curved forceps seem most useful) by placing them under the entire brain and pulling in an upwards motion.

Sample collection

Normal diagnostic:

Collection of kidney, liver, spleen, thymus, gill, muscle, brain, blood, lesions and intestine.

Specialised sampling

- **Bacteriology:** Take the relevant samples.
- **Electron Microscopy:** Take the indicated samples.

Sampling tissues from a finfish post mortem - for histology

Introduction

The essential requirements for sampling are:

- That the specimen must be placed in the chemical fixative as quickly as practicable (especially the gill tissue, as this begins to alter several minutes post death) and ensure that the tissues have not been previously frozen.

  Note the elapsed time of death, if known, and the state of the sample on the work sheet.

- Specimens are in sufficient fixative (10:1 v/v or greater).
- Specimens are handled gently to prevent artefacts and damaging architecture.
- That the tissues are trimmed to dimensions of 10 mm by 3 mm thick. Small fish can be fixed whole, provided a longitudinal slit is made in the abdominal cavity to expose internal organs to the fixative.

Lesions

When sampling gross lesions select a piece of tissue that includes normal and affected zones.

Decalcification

Tissue samples containing calcium in the form of bone or scales should be fixed, trimmed and then submitted to the Histology section with a note to decalcify prior to processing.

Skin with scales- cut oversize, decalcify and then trim to size for histology (scales are easily dislodged by cutting in fresh material)

Fixatives

The fixatives recommended are 10% neutral buffered formalin or 10% formalin in seawater.

Sampling tissues from a finfish post mortem for Bacteriology

Introduction

The three essential requirements for sampling are:

- Blood and swab samples should be taken aseptically.
- That bacteriological isolation is dependent on the correct choice of growth media. This choice is determined by the environment from which the specimen was taken (temperature, salinity etc.).

Samples submitted for bacterial culture

Blood sampling

The removal of blood from circulation can be done in several ways: truncation of the caudal peduncle (tail), heart puncture, caudal vessel puncture, *ductus Cuvieri* puncture and repeated sampling via chronic cannulation of the aorta. Refer to Figure 1.

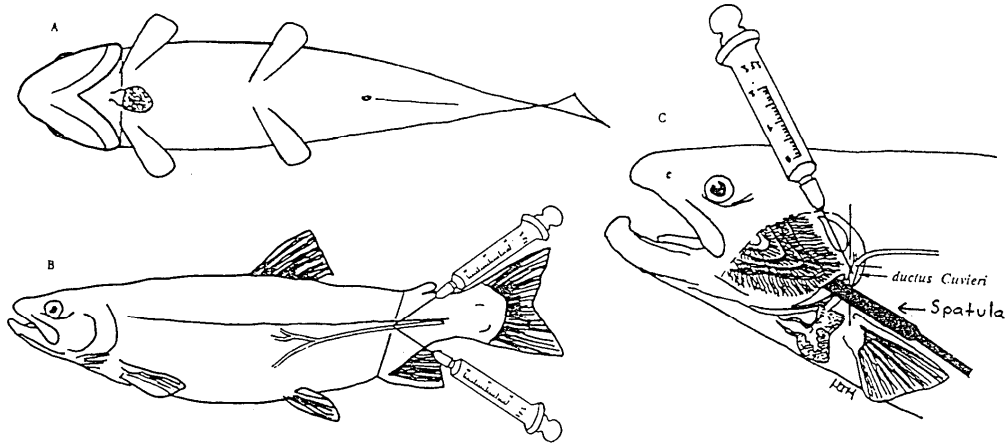

Figure 1. Common sites for obtaining blood samples from fish: A, heart; B, puncture of the caudal vessels; C, puncture of the Ductus Cuvier.

Destructive sampling

Blood may be collected via any method after ensuring that the area of puncture is flooded with 70% ethanol. The most favoured site are the caudal vessels or the *ductus Cuvieri* (Figure 1). Caudal truncation is usually only performed on small fish since the finer anatomical features render other techniques less efficient. Cardiac puncture is most easily performed by exposing the heart so that needle placement can be precise.

Non–Destructive sampling

If a blood sample only is required, the sacrifice of the fish is not necessary. The use of effective non–lethal doses of anaesthetics and a puncture technique allow the sampled fish to be returned to the population upon recovery.

The choice of sampling site is influenced by several considerations: the thickness of the overlying tissue, the ease of exact location of the site from anatomical landmarks and the potential for damage to the vessels and associated tissues.

The site of choice is the puncture of the caudal vessels. See Figure 1.

Liver

A slice of liver is submitted at least 3 mm thick, enabling the sample to be flooded with ethanol and flamed before a swab is plunged in the centre. Alternatively, place the sample into a Petri dish.

Spleen

A slice of spleen can be sampled in the same way as the liver described above.

Lesion

Lesions can be cut from the animal and treated the same as other tissues, taking a swab sample or in the case of a skin lesion. With small fish the whole animal can be flooded with ethanol, flamed and a swab plunged into the lesion.

Kidney

A slice of kidney can be removed, and treated in the same way as the liver sample mentioned above. The preferred method for sampling is to pierce the swim bladder and flood with ethanol. Remove the excess ethanol by rolling the liquid off the site of puncture. Puncture the kidney on a dorso-ventral angle, drawing both blood and tissue. The anatomy of the kidney will depend on the species of fish being sampled. The site of puncture should be chosen to have the largest tissue mass. Smaller fish can be sampled by cutting anterior to the caudal peduncle, (ensuring that the abdomen stays intact) flooding the cut surface with ethanol and placing the needle directly into the kidney.

Samples submitted for fungal culture

If swabs are to be taken for fungal culture of infected sites, then this must be done prior to any of the abovementioned aseptic techniques.

Appendix 3. Database of Results of Submissions (Sample Worksheet)

Lab reference	Date collected	Senders reference	Date received	Country of origin	Common name	Genus	species	mortality rate	Day of quarantine	Diagnosis	Comments

Appendix 4: Number of Cases Submitted by AQIS Inspectors, to the State Laboratory, Western Australia by Disease Agent and Calendar Year

	1999	2000	2001	2002	2003	2004	2005
Number of AQIS fish submissions	12	6	7	15	12	16	13
Diagnoses							
Viral -specify eg.							
Goldfish Herpesvirus Haematopoietic Necrosis						1	
Spring Viraemia Carp Virus							
Iridoviruses			1	2		1	
Rhabdovirus carpio							
IPNV							
Lymphocystis	1						
Renal Necrosis suggestive of viral infection	1						
Kidney Spleen Liver necrosis ~ Viral Infection			1	4	1		
Pancreatic Necrosis ~ viral			1				
Bacterial - specify							2
Aeromonas salmonicida							
Edwardsiella tarda							
Chlamydia/Rickettsia	1						
Gram Negative Rods	1			1			
Aeromonas hydrophila	3	1		3	5	2	4
Mycobacterium marinum	1						1
Pleisiomonas shigelloides	1						
Streptococcus sp.(non-iniae)				3		1	
Streptococcus iniae			1				
Flavobacterium columnaris				1	1	1	
Vibrio alginolyticus					1	1	
Vibrio harveyi							1
Parasitic							
Protozoans -Ciliates							
Chilodonella	1		1				1
Tetrahymena						2	
Uronema							
Ichthyophthirius						1	1
Trichodina							
Protozoans - Amoeba							
Sphaerospora-like	1	2					
Protozoans - Flagellates							
Oodinium					1	1	
Ichthyobodo (Costia)		1					1
Cryptobia			2		1	2	1
Protozoan unidentified				1		1	
Microsporidia							2
Myxosporidian							
Muscle myxosporidian	1	1				1	
Myxosporidian peritoneal granulomas						1	

Appendix 4 : AQIS submissions to the Western Australian laboratory prior to this project

Trematodes - Monogeneans					
Gyrodactylus (marine)					1
Dactylogyrus				1	2
Nematodes – specify					
Digeneans – specify					
Cestodes – specify					
Crustaceans – specify					
Fungal					
Saprolegnia					
EUS	1		1		1
Basidiobolus			1		
Water Quality Problems		1			1
Cyanide Capture				1	

Appendix 5: Number of Ornamental Fish Cases Submitted by Non-AQIS Sources to the State Laboratory, Western Australia by Disease Agent and Calendar Year

	1999	2000	2001	2002	2003	2004	2005
Number of Non-AQIS fish submissions	9	13	15	12	9	9	20
Diagnoses							
Viral -specify eg.							
Goldfish Herpesvirus				2	1	1	3
Haematopoietic Necrosis							
Spring Viraemia Carp Virus							
Iridoviruses							
Rhabdovirus carpio							
IPNV							
Lymphocystis			1				
Renal Necrosis suggestive of viral infection							
Kidney Spleen Liver necrosis ~ Viral Infection							
Carp Pox (herpes virus)						1	
Pancreatic Necrosis ~ viral							
Bacterial – specify							1
Aeromonas salmonicida							
Edwardsiella tarda							
Chlamydia/Rickettsia							
Gram Negative Rods	1	3					
Aeromonas hydrophila			1	2			3
Mycobacterium marinum	1		1	1		1	
Pleisiomonas shigelloides							
Streptococcus sp.(non-iniae)							
Streptococcus iniae							
Flavobacterium columnaris		1			2		
Vibrio alginolyticus	1				1		
Shewanella putrifaciens							1
Parasitic							
Protozoans –Ciliates							
Chilodonella		1					
Tetrahymena		1		1			
Uronema			1				
Ichthyophthirius	1[1]	1	2		1		2
Trichodina			2				
Protozoans - Amoeba							
Sphaerospora-like							
Protozoans - Flagellates							
Oodinium			1				
Ichthyobodo (Costia)		1		1		2	2
Cryptobia			2	1	2	1	
Protozoan unidentified	1 kidney possibly						

[1] Cryptocaryon

Appendix 5: Ornamental fish submissions to the Western Australian laboratory prior to this project

	Trimena sp.						
Microsporidian unidentified						1	
Myxosporidian							
Muscle myxosporidian - Pleistophora like		1				1	
Myxosporidian peritoneal granulomas							
Trematodes - Monogeneans							
Gyrodactylus (salt)					1		
Dactylogyrus	1	2	1	1			1
Nematodes – specify							
Digeneans – specify							
Cestodes – specify							1
Crustaceans – specify							
Fungal							
Saprolegnia			1				
EUS			1				
Basidiobolus							
Neoplasia	3	1	1	2	1		
Water Quality Problems		1				1	7
Cyanide Capture							
Submitted as "Dropsy" - cause not determined			1	1			

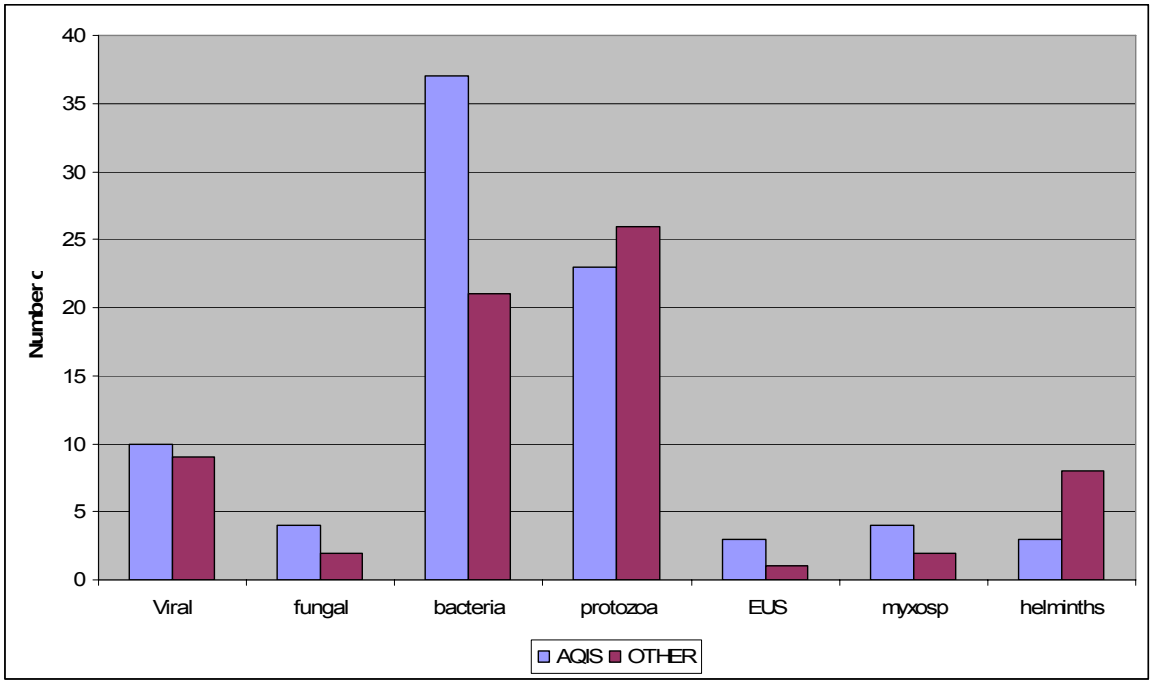

Figure 4. Breakdown of cases submitted in Western Australia, 2000-2005, by category. Myxosp = myxosporidians, EUS = Epizootic ulcerative syndrome.