



CEBRA Report Cover Page				
Project Title, ID & Output	<i>1305A Ornamental Fish Import Reform – Surveillance Model; Outputs 2 and 3</i>			
Project Type	<i>Report</i>			
DAFF Project Sponsor	Andrew Cupit	DAFF Project Leader/s	Yuko Hood	
CEBRA Project Leader	Andrew Robinson	NZ MPI Collaborator	Brian Jones	
Project Objectives	<p><i>This project will design and test an on-arrival health surveillance (monitoring) system that is a key component of the overarching Ornamental Fish Import Reform (OFIR) project, which proposes to change the way the Department of Agriculture (hereafter, the Department) manages risks associated with the importation of ornamental fish. Ornamental fish is the only live animal commodity that is routinely imported in large numbers into Australia.</i></p> <p><i>The Department’s import controls for the ornamental fish pathway have been subject to longstanding criticism by state governments, the aquaculture industry, and academia. In-house and independent reviews have identified significant limitations in the import controls, which are based largely on detaining fish post-arrival in quarantine-approved premises. It is vital that the Department introduces more effective measures in order to better meet its obligations under the Quarantine Act, as well as to be consistent with the conservative approach that the Department takes in managing risks associated with the importation of other live animal commodities.</i></p>			
Outputs	<p>2) <i>Data analysis and evaluation for trial phase 1.</i></p> <p>3) <i>Design of trial phase 2</i></p>			
Original Budget	<i>Original budget is listed within the CEBRA Workplan.</i>			
	Year 2013-14	Year 2014-15	Year 2015-16	Year 2016-17
	<i>CEBRA \$50,000</i>	<i>CEBRA \$50,000</i>	<i>TBA \$0,000</i>	<i>TBA \$0,000</i>
Project Changes	<i>NIL</i>			
Research Outcomes	<p><i>The report provides a statistical summary of the outcomes of trial phase 1.</i></p> <p><i>Achievement of policy objectives — the risk-based testing follows the government’s broader biosecurity reform agenda and aims to shift the emphasis of risk management off shore. This testing program will monitor the effectiveness of overseas authorities in ensuring the health of imported ornamental fish. Importantly, this work will benefit the Department by providing a means by which it can continue to facilitate imports and at the same time meet its obligations under the Quarantine Act.</i></p> <p><i>The deliverables from this project will contribute significantly to the development of a unique import health monitoring system that will facilitate the safe importation of live ornamental fish to Australia. This project will develop close relationships with state-run services and form new collaborative partnerships in biosecurity intelligence.</i></p>			
Recommendations	<i>The department and CEBRA continue to finetune the fish monitoring system including the analysis of Phase 2 data, possibly expanding the scope of the trials and holding workshops to broaden the basis of the expert elicitations.</i>			
Related Documents	<i>Project Business Case</i>			

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Risk-Based Management for Imported Ornamental Fish.

CEBRA 1305A Report

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Executive Summary

This report summarises the development of the on-arrival ornamental fish health monitoring system. The system is central to the Department of Agriculture (the department)'s ornamental fish import reform project, under which the department proposes to change the way it manages the biosecurity risks associated with the importation of ornamental fish into Australia.

The ACERA II 1206G Ornamental Finfish Import Reform Program developed a sampling framework and trial for the surveillance program that focused on trialing the operational feasibility of the monitoring system using non-compliant bags only (Phase 1). The data generated from Phase 1 testing are analysed in this report. Australian Import Management System (AIMS) data for all ornamental fish consignments for a 12 month period were analysed to provide a snapshot of activity for various pathways. A pathway is defined as all consignments in a particular risk group of fish imported by an importer and supplied by an exporter. The findings of these analyses are proved in this report.

This report also describes the proposed design of the next trial (Phase 2) of the fish health monitoring system. This trial has two components:

- A. Sampling, processing and testing of fish from **compliant fish bags**, i.e. bags of imported ornamental fish that on inspection are determined to be visually healthy.
- B. Sampling, processing and testing of fish from **non-compliant fish bags**, namely bags of imported ornamental fish that on inspection are determined to be associated with substantial signs of communicable disease.

For compliant bags, CEBRA proposes the use of sampling algorithm from the Continuous Sampling Plan (CSP) family. Similar algorithms have already been trialed for several imported plant-products. Each pathway is assigned to an 'enhanced' mode (a higher frequency of sampling) or 'monitoring' mode (a lower frequency of sampling), depending on the history of compliances of that pathway. The sampling framework will be effected through a dashboard, a spreadsheet-based program, developed by the department, which is linked to AIMS. The dashboard will automatically consider each imported consignment in the context of the pathway it represents and direct departmental officers to sample fish, based on a CSP algorithm specifically designed for ornamental fish.

For non-compliant bags, there is the need to develop definitions of non-compliance for inspectors, and a histological index that standardises the reporting of laboratories' findings. This involves estimating disease levels associated with the occurrence of different kind of gross symptoms and histopathological observations. The main objective of this component of the monitoring system is to better identify emerging disease risks. CEBRA proposes to use an expert elicitation methodology to develop a definition of non-compliance and to develop the histological index.

It is recommended that the department and CEBRA continue to fine tune the fish monitoring system including the analysis of Phase 2 data, possibly expanding the scope of the trials and holding workshops to broaden the basis of the expert elicitations.

Acknowledgments

The authors are grateful to Ms. Jillian Mitchell and Dr. Ramesh Perera for their considerable investment and contribution to the research project documented here. Ms. Jessica Holliday was greatly involved in preliminary work of the project, and with the recent assistance of Dr. Tracey Hollings, is responsible for Figure 1.1. The Department of Agriculture officers working at the South East Regional Office have been very supportive of the many requests made to them. Finally, the authors thank two anonymous reviewers and Professor Colin Wilks, the chair of the CEBRA Scientific Advisory Committee for their detailed and constructive review comments.

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Introduction

1.1 Background

Australia's current policy for importation of ornamental fish does not fully address the risks associated with subclinically¹ infected fish. The Secretary of the Department of Agriculture agreed on 22 December 2011 to support the development of an on-arrival fish health monitoring and testing program that would monitor the effectiveness of overseas authorities at exporting to Australia consignments of healthy ornamental fish, free of subclinical infection with specific diseases² of quarantine concern. The shortcomings of the current arrangements are highlighted by substantial disease incursions in recent times, including the establishment of goldfish herpes virus and the detection of gourami iridovirus in imported fish post-quarantine in Australia.

For ornamental fish, the large number of animals (in the millions), the vast range of host fish species and large number of countries from which they are sourced complicates the risk and options for risk management compared to most imported animal commodities. The disease risk is attributable to a wide range of known disease agents and the periodic emergence of new aquatic animal diseases that may be carried in ornamental fish.

Australia has a stringent biosecurity policy for importation of ornamental fish that includes pre-border health certification and a mandatory minimum quarantine period of one to three weeks (depending on the species) in registered quarantine-approved premises (QAPs). While these standards aim to mitigate the risk of pathogen incursion, they have failed to prevent some disease incursions, for example the establishment of goldfish herpes virus and the periodic detection of megalocytiviruses and *Edwardsiella ictaluri* in imported fish.

Department of Agriculture Animal Biosecurity and Animal Import Operations branches completed a review of current post-arrival import conditions for ornamental fish and concluded that the current post-arrival quarantine arrangements, based on an isolation period at privately owned QAPs, are not fully effective. Importantly, the review found that the current system does not address risks associated with subclinically infected fish or risks associated with emerging diseases.

In recognition of this problem and in an attempt to better manage risk, the Department is proposing changes to the way it manages the disease risks associated with imported ornamental fish. The changes include on-arrival risk-based testing to monitor

¹Meaning, non-clinical infection with an infectious disease agent.

²Disease refers to clinical or non-clinical infection with one or more of the aetiological agents of the disease.

the performance of overseas authorities and exporters in meeting the health requirements for ornamental fish exported to Australia. As well as testing directly for the presence of pathogens that are of concern to Australia, the proposed system will make more use of epidemiological evidence that suggests the presence of potentially important but unidentified or unconfirmed disease agents.

1.2 Approach

The ongoing ability of the overseas competent authorities to comply with Australia's import requirements and thereby ensure the health of fish exported to Australia will be monitored by an on-arrival health monitoring system. The monitoring system must be sufficiently sensitive to detect systematic failures on the part of the competent authority. Evidence collected through the monitoring system will form the basis of the department's response to detected non-compliances, including suspension of imports if non-compliances are not satisfactorily resolved.

The information generated by the monitoring system will be quantitative and semi-quantitative in nature, and will be used in conjunction with operating guidelines to determine non-compliance by overseas authorities. Ongoing analysis of the monitoring data may be supported by ABARES.

At the core of the department's new approach will be a strengthening of pre-export requirements that overseas competent authorities ensure and attest that exported fish are being sourced from populations that are free of specific disease agents, coupled to on-arrival monitoring of imported fish to ensure on-going compliance by overseas authorities. Under the World Organization for Animal Health (OIE) guidelines, a competent authority can maintain a zone or compartment disease-free status by operating a surveillance program that can detect the specific disease agent with 95% confidence when at a prevalence of 5% or more, after two years of demonstrated freedom.

1.3 Fish Health Monitoring System

Previous ACERA work has proposed the use of the Continuous Sampling Plan (CSP) family of random sampling algorithms for several imported plant-products pathways, see Robinson et al. (2012) and Robinson et al. (2013). CSP involves choosing a sub-pathway level of intervention, such as disease class and/or competent authority, and keeping separate inspection histories for each sub-pathway.

This classification will provide the basis for distinguishing two sample testing protocols, which are summarised in Table 1.1. Consignments can be non-compliant due to signs of disease during inspection, but also for other reasons such as incorrect documentation or the presence of animal or plant contaminants.

The mechanics of the proposed monitoring system (Figure 1.1) include:

- all shipments of freshwater and marine ornamental fish will continue to be visually inspected on arrival; based on visual inspection, the bags will be classified as compliant or non-compliant.
- Compliant bags are handled using the protocol in Section 1.3.1, briefly summarized here.

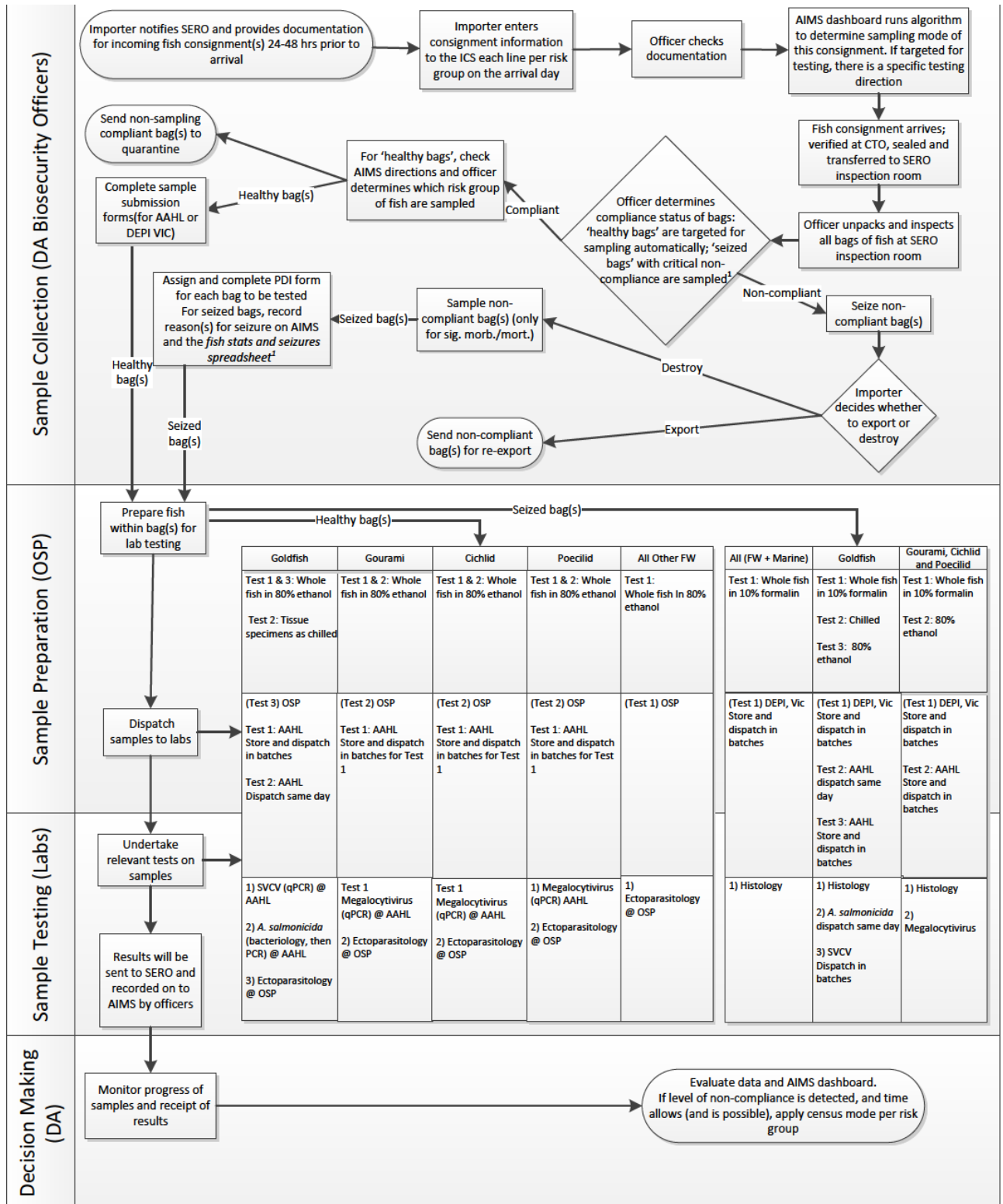


Figure 1.1: Overview of proposed monitoring system.

- Bags of fish in compliant consignments will be subject to random sampling during on-arrival inspection, using a CSP algorithm, before being released.
- CSP sampling will be based on type of disease agent, risk group of fish, and/or competent authority, exporter, or importer (Section 1.3.1)
- Non-compliant bags are seized as per the protocol in Section 1.3.2, briefly summarized here.
 - bags of fish in consignments that show substantial mortality or morbidity as determined by the work instruction (Appendix A) will be sampled during on-arrival inspection and subject to general histopathological analysis, and the remaining fish destroyed or re-exported (Section 1.3.2).
- In general, fish samples will be sent to participating laboratories for testing for specific disease agents of biosecurity concern;
- the information obtained from the samples and from border inspection will be analysed by Animal Biosecurity Branch of the Department of Agriculture (the pathway manager), possibly with support from ABARES;
- and appropriate action taken to manage any biosecurity risk at the source.

Table 1.1: The two sample testing streams of the surveillance program

	Protocol 1 (Compliant)	Protocol 2 (Non-compliant)
Aim	Monitor the prevalence of pathogens of biosecurity concern entering Australia in fish	Monitor the general health of non-compliant fish exported to Australia.
Source	Randomly selected consignments from all compliant consignments	All non-compliant consignments
Testing	Virus PCR, bacteriological, and parasitological testing for pathogens of biosecurity concern, which are currently: <i>A. salmonicida</i> , SVCV and Megalocytivirus, and ectoparasites of biosecurity concern	Histopathology for general health, bacteriology, and virus PCR testing.
Testing Unit	One bag of fish (fish counts per bag vary from one to hundreds depending on species and supplier)	One bag of fish (fish counts per bag vary from one to hundreds depending on species and supplier)
Sample Design	Risk-based fish selection	Targeted sampling, i.e., biased towards the unhealthiest fish.

The pathway is defined as all consignments containing species of fish that are in a particular risk group imported by an importer and supplied by an exporter in an exporting country. A pathway describes the potential entry route of a disease into Australia. The numbers of possible pathways this sampling model may need to describe and monitor are numerous, with 26 countries, five different risk groups of fish (only freshwater fish will

be sampled, so marine species are excluded from the Compliant Bags protocol below), around 50 importers, and more than 200 exporters.

Definitions of compliant and non-compliant bags, and guidelines for laboratories to report using standardised histological indices, are being developed using a suite of expert elicitation workshops. An expert elicitation workshop to progress development of a histopathology index was held at the Department's South East Regional Office on 6–7 August 2014. This involved estimating disease levels associated with the occurrence of different kinds of gross symptoms and histopathological findings, in order to better identify emerging disease risks. The department/CEBRA proposed to use expert judgment in such circumstances when decisions are imminent and the relevant data are unavailable. The particular approach proposed for use in the assessment employed expert groups, structured question formats, independent estimates, and iteration of the estimation process to generate expert estimates of facts that are relatively well calibrated and accurate. Additional workshops with wider expert participation are planned.

1.3.1 Compliant Bags Protocol

The Compliant bags protocol follows a CSP algorithm (Robinson et al., 2012). Usually, the pathway begins in *enhanced* mode, so samples are taken from all, or an large number, of compliant consignments for laboratory testing. For example, compliant bags from the first ten consignments of the pathway would be sampled for laboratory testing according to the standard protocol. If all ten consignments are deemed compliant, then the pathway is moved into a *monitoring* mode, and consignments are all inspected visually as before, but are also sampled randomly for lab testing, say one in ten, or one in twenty. That is, in monitoring mode, samples of compliant bags are taken from 10% or 5% of the consignments, selected randomly. If consignments from a pathway reach a trigger level of pathogen detection by the laboratory (e.g., 2 consecutive positive consignments), then that pathway returns to (or remains in) enhanced mode, meaning that consignments are again sampled for laboratory testing for a specific disease agent at an increased rate, until a predetermined number (say ten) of successive consignments are found to pass (i.e., test negative). Note that this description of the algorithm is an example, and the decision of whether to start the sub-pathway in enhanced mode, what sampling rates to use in each mode, and how long to remain in enhanced mode, should all be set as a matter of policy. The design of surveillance would provide a 95% confidence level of detecting a disease agent if it were present at a prevalence of 5% or more. The laboratories participating trials of surveillance should assess each test's diagnostic sensitivity and specificity.

1.3.2 Non-Compliant Bags Protocol

Bags that are deemed non-compliant will be seized. Samples will be taken by border staff and sent to a laboratory for testing. The testing will include histopathology for general health, bacteriology, and virus PCR testing.

1.3.3 Trial Phase 2

The second and any subsequent phases of this trial form the basis of this CEBRA biosecurity research project idea, and will:

- comprehensively test and refine the sampling framework, including its capacity to be modified to address changing risks and circumstances,
- comprehensively test and refine the operational systems for sample collection, transport and data entry,
- develop and test a histology based health index of general fish health, using weights provided by expert elicitation or point-of-truth calibration (POTCAL); and
- design a framework for data analysis.

The sampling framework is implemented as the ornamental fish dashboard, a spreadsheet-based program, developed by the Department of Agriculture. The dashboard is linked to the Australian Import Management System (AIMS) and the Integrated Cargo System that manages imported cargo, and automatically selects consignments for sampling based on the ornamental fish specific CSP algorithm. This report provides analysis and evaluation of trial phase 1, and design principles for trial phase 2.

2

Phase 1 Analysis

2.1 Introduction

Phase 1 of the project involved data capture for all live fish consignments (Section 2.2) and lab testing (Section 2.4) of sampled fish in the South-East Regional Office (SERO). The design of Phase 1 is reported in Halliday and Robinson (2013). Phase 1 of the trial began at SERO on 6 May 2013 and lasted until the end of July 2013.

Phase 1 of the trial was restricted to Stream 2 sampling (see Section 1.3). Its main purpose was to test the operational feasibility of the data collection, sample collection, sample preparation, storage, transport and laboratory testing elements of the program. In this case, statistical input to the sampling framework was not critical for this project.

To test the operational characteristics of the sampling framework in this first phase of the trial, we targeted only those bags of fish that would otherwise be destroyed due to non-compliance with current Australian import requirements (Appendix A).

2.2 Data Summary

For the following analysis, a *consignment* refers to a single quarantine entry. Each combination of quarantine entry, species (using the recorded scientific name), and exporter is considered a unique *line*. Before the trial, the identity of lines was based on the use of tariff codes to distinguish marine and freshwater fish. With the implementation of the new system, the line will be based instead on risk group. In general, the rigour of data entry by brokers on the ornamental finfish pathway is not as high as that of other pathways.

Figure 2.1 provides a snapshot of the activity on the pathway, showing histograms of the number of lines per consignment, the number of tails per consignment, and the number of tails per line. Table 2.1 presents the country-level summary of the arrivals at SERO during the trial period. Table 2.2 presents the summary of the arrivals by risk group. Table 2.3 presents the summary of the arrivals by importer, for selected high-volume importers. Table 2.4 presents the summary of the arrivals by supplier, for selected high-volume suppliers.

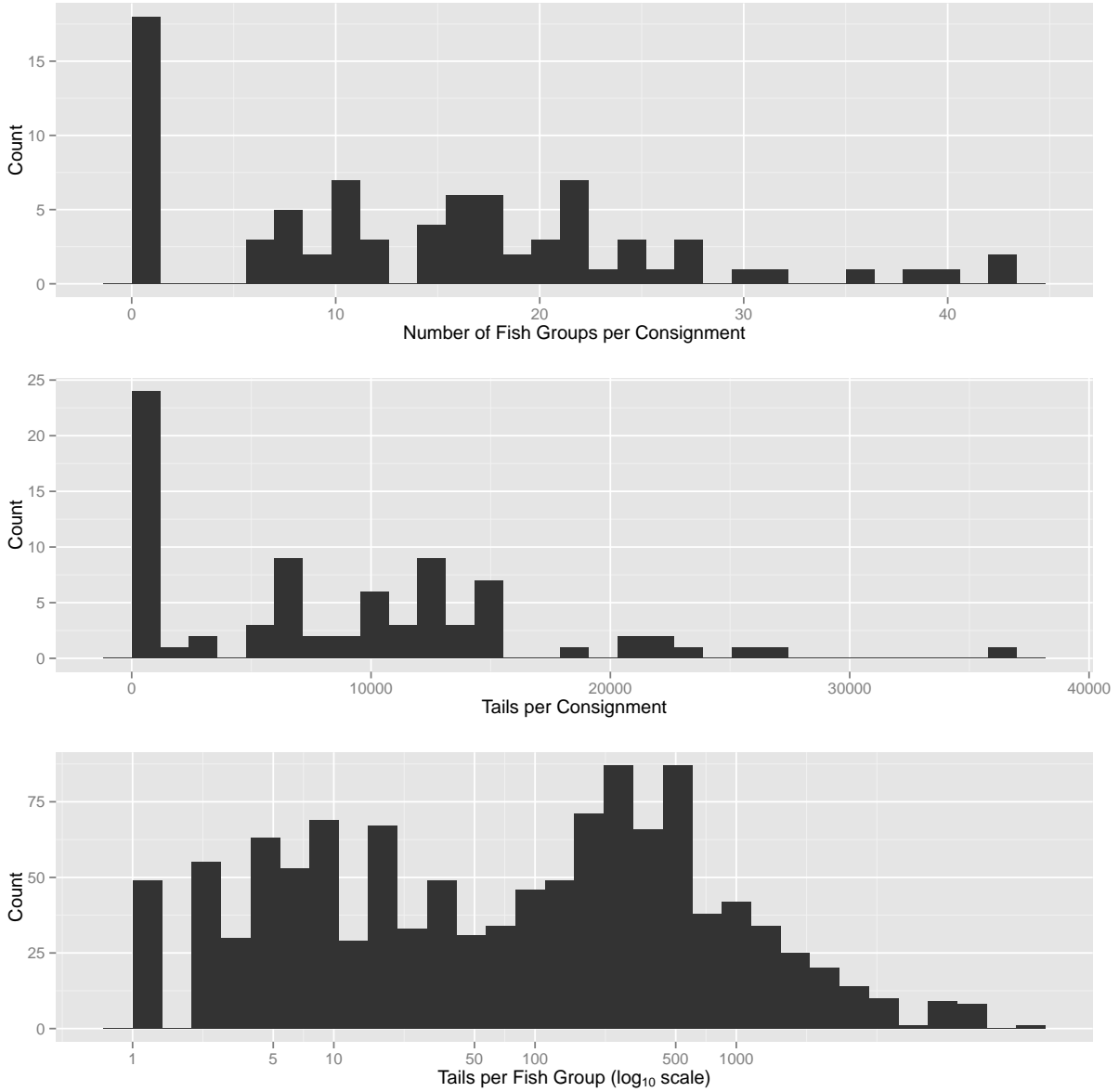


Figure 2.1: Summary plots of arrival statistics by fish group for Phase 1. The top panel provides number of fish groups (here, species) per consignment, the middle shows the number of tails per consignment, and the bottom panel shows the number of tails per fish group (here, species). The data are all arrivals at SERO from 6 May 2013 until July 31 2013.

Table 2.1: Summary statistics by country for ornamental finfish from Phase 1, namely, all ornamental finfish entering the Melbourne Regional Office from 6 March to 6 December 2013, inclusive. *Entries* is the number of consignments, by which the table is ordered. *Ktails* is the number of thousands of tails, and *Morbid* and *Dead* are the numbers of sick and dead fish, respectively. *Fam* and *Grps* report the number of families and the number of risk groups exported from the country, respectively. The *Supp* and *Imp* columns report the number of suppliers and importers that have exported from each country during the time period.

Country	Entries	Bags	Ktails	Morbid	Dead	Grps	Fam.	Supp.	Imp.
INDONESIA	29	10004	243.4	12	66	6	29	10	5
SINGAPORE	16	1018	155.5	6	57	5	8	6	4
MALAYSIA	9	744	103.6	0	122	5	6	3	2
SRI LANKA	9	246	107.5	0	8	1	1	2	2
THAILAND	7	222	49.8	0	143	5	7	2	2
CHINA	4	135	32.5	0	67	1	1	2	1
GERMANY	4	107	5.3	0	136	2	2	3	2
USA	1	262	0.3	3	1	1	5	1	1
VANUATU	1	206	0.2	1	1	1	7	1	1

Table 2.2: Summary statistics by risk group for ornamental finfish from Phase 1, namely, all ornamental finfish entering the Melbourne Regional Office from 6 March to 6 December 2013, inclusive. *Cties* reports the number countries exported from in the risk groups. See Table 2.1 for the other column definitions.

Risk Group	Entries	Bags	Ktails	Morbid	Dead	Cties	Fam.	Supp.	Imp.
Other Freshwater	47	2590	296.1	0	325	6	7	15	6
Goldfish	44	877	166.2	6	129	5	1	12	4
Poeciliid	33	666	172.5	0	32	5	3	10	3
Gourami	32	395	42.1	0	46	4	2	11	4
Cichlid	31	365	12.7	0	31	5	2	15	6
Marine	20	8053	8.1	16	38	3	22	9	5

Table 2.3: Summary statistics by importer for ornamental finfish from Phase 1, namely, all ornamental finfish entering the Melbourne Regional Office from 6 March to 6 December 2013, inclusive. See Table 2.1 for the column definitions.

Importer	Entries	Bags	Ktails	Morbid	Dead	Cties	Fam.	Grp.	Exp.
a	62	9371	620.21	17	512	8	30	6	15
b	8	1400	56.82	3	34	4	22	6	6
c	3	1096	1.18	2	7	2	18	1	3
d	2	649	0.65	0	3	1	17	1	1
e	1	2	0.00	0	0	1	1	1	1
f	1	66	3.55	0	41	1	4	4	1
g	1	25	0.36	0	0	1	2	2	1
h	1	52	14.50	0	4	1	6	5	1
i	1	284	0.28	0	0	1	11	1	1
j	1	1	0.28	0	0	1	1	1	1

Table 2.4: Summary statistics by supplier for the top 20 suppliers for ornamental finfish from Phase 1, namely, all ornamental finfish entering the Melbourne Regional Office from 6 March to 6 December 2013, inclusive. See Table 2.1 for column definitions.

Supplier	Entries	Bags	Ktails	Morbid	Dead	Cties	Fam.	Grp.	Imp.
a	8	226	102.00	0	8	1	1	1	1
b	8	1362	197.08	0	24	1	7	5	1
c	7	662	99.52	0	81	1	5	4	1
d	7	738	74.94	3	15	1	7	5	1
e	6	176	44.23	0	143	1	6	4	1
f	6	2999	3.69	9	5	1	16	2	1
g	5	158	52.63	0	35	1	7	5	2
h	5	2487	2.49	2	18	1	14	1	1
i	4	112	28.88	0	65	2	1	1	1
j	2	74	4.80	0	136	1	2	2	1
k	2	649	0.65	0	3	1	17	1	1
l	2	488	0.49	0	4	1	15	1	1
m	2	750	31.36	0	2	1	8	5	1
n	1	2	0.00	0	0	1	1	1	1
o	1	284	0.28	0	0	1	11	1	1
p	1	52	6.94	3	3	1	6	5	1
q	1	66	3.55	0	41	1	4	4	1
r	1	95	6.34	0	4	1	4	3	1
s	1	1	0.28	0	0	1	1	1	1
t	1	445	0.45	0	0	1	16	1	1

2.3 Bag by Bag

This section presents the bag-by-bag failure rates. Table 2.5 reports the total number of bags by risk group, along with the number of bags that have any morbid or dead fish upon inspection. Further analysis is complicated by the fact that data capture was ambiguous; for a number of lines (as defined above) we have the number of bags, and the number of dead fish, but we do not know how the dead fish were distributed amongst the bags.

Table 2.5: Bag-level statistics by risk group. The *Morbid* and *Dead* columns report the number of bags with at least one morbid or dead fish respectively.

Risk Group	Total	Morbid	Dead
Cichlid	365	0	15
Goldfish	877	2	48
Gourami	395	0	18
Poeciliid	666	0	15
Other Freshwater	2590	1	45
Marine	8053	9	29
Total	12946	12	170

2.4 Laboratory Results for Trial 1

The Department is undertaking a research trial of the on-arrival fish health monitoring system to test its operational feasibility. The design of the first phase of this trial was described in the ACERA II (Project 1206G) report. It focused on the development and operational feasibility of the monitoring system, including sample collection and shipment, and data entry at one port (Appendix B).

Trial 1 was conducted as per work instructions (Appendix B). The information on the diagnostic sensitivity and specificity of the diagnostic test used for Trial 1 are not available. The laboratory testing was intended to test only those bags of fish that would otherwise be destroyed due to non-compliance with current Australian import requirements. Broadly, non-compliance referred to a failure of the goods to pass inspection or the documentation verification (or both) determined by inspectors on-arrival at the border due to at least one of the following conditions.

- Fish not certified.
- Incorrect species on invoice.
- Non-permitted species.
- Unidentified species.
- Undersized fish.
- Insect in bag.
- Snails in bag.
- Eggs in bag.
- Plant material in bag.

- Coral in bag.
- Signs of disease, namely
 - Substantial mortalities in a bag,
 - Loss of colouring or darkening,
 - Abnormal swimming behaviours, hovering at the surface,
 - Lethargy or hyper/hypo-activity,
 - Fin burn/congestion/erosion,
 - Abnormal respiratory activity such as rapid gulping,
 - Loss of balance, swimming on the side,
 - Excess mucous secretion,
 - Abnormal swelling,
 - Bulging eyes,
 - Reddening/hemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes, and
 - Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss.

During Phase 1 of the trial, no bags were non-compliant due to signs of ill health. Therefore, some bags of fish were purchased for lab testing so that the operational feasibility of sampling and transportation could be assessed. The test results, presented in Appendix D and summarised in Table 2.6, included non-compliant fish (other than disease issues) and purchases of healthy fish. The test results show that all the tested (purchased, healthy) goldfish were free from bacteria and viruses of concern, each risk group had some failures for histology, and gourami and poeciliid both had some fails for viral testing. The majority of the goldfish histology test results did not show specific histological problems.

Table 2.6: Summary of lab testing results for Phase 1. Three test types are reported, namely bacterial testing, histological testing, and viral testing. Results are organized by risk group. All results are *Pass* or *Fail* except for the histological tests, some of which were uncertain (*Unc.*).

Risk Group	Bacteria			Histology				Virus		
	Fail	Pass	Total	Unc.	Fail	Pass	Total	Fail	Pass	Total
Purchased										
Goldfish	0	21	21	7	2	3	12	0	12	12
Gourami	0	0	0	0	1	6	7	2	6	8
Other	0	0	0	0	0	0	0	0	0	0
Poeciliid	0	0	0	0	1	7	8	2	7	9
Seized										
Goldfish	0	0	0	0	0	0	0	0	0	0
Gourami	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	2	1	2	5	0	0	0
Poeciliid	0	0	0	0	0	2	2	0	2	2
Total	0	21	21	9	5	20	34	4	27	31

3

Phase 2 Planning

The design of Phase 2 trial has described in Section 1.3. Phase 2 of the trial will begin at SERO in May 2014 for two weeks then again from June to August 2014 for 8 weeks. The detailed procedures are described in the work instructions (Appendix C). The flow chart is shown at Figure 1.1. The purpose of this chapter is to plan for Phase 2, rather than to provide information about the ornamental finfish pathway at the national level. Hence our focus is on SERO.

3.1 AIMS Data Summary

This section provides a statistical overview of the AIMS data for ornamental fish imports provided by the Department. The full AIMS dataset comprises 7692 quarantine entries (consignments) with record creation dates ranging from January 2008 to November 2012, and comprises consignments from 21 countries, 155 importers, and 288 exporters.

The following tables describe the basic characteristics of the pathway. The statistics in Table 3.1 summarize the AIMS data by country. Table 3.2 summarizes the inspection data for suppliers. Table 3.3 summarizes the inspection data for importers.

Table 3.1: Summary statistics by country for ornamental finfish. *Consignments* is the number of consignments, by which the table is ordered. *Total* is the number of tails, *DOA* are the count and percentage of tails dead upon arrival, and *Deaths* are the count and percentage of tails that die in post-entry quarantine. The *Suppliers* and *Importers* columns report the number of suppliers and importers that have exported from each country during the time period.

Country	Consignments	Total	DOA	DOA (%)	Deaths	Deaths (%)	Suppliers	Importers
INDONESIA	2193	21,354,308	638,496	3.0	1,045,091	4.9	137	94
SINGAPORE	1306	14,240,116	165,876	1.2	647,685	4.5	70	56
THAILAND	732	5,588,118	363,305	6.5	281,145	5.0	58	33
MALAYSIA	651	5,134,108	103,119	2.0	338,240	6.6	77	47
SRI LANKA	592	5,120,340	79,758	1.6	179,806	3.5	49	42
CHINA	485	5,593,125	145,311	2.6	361,210	6.5	73	32
VANUATU	300	194,396	12,505	6.4	12,156	6.3	42	27
PHILIPPINES	253	198,465	14,299	7.2	24,198	12.2	44	35
USA	171	147,817	11,845	8.0	13,234	9.0	31	26
GERMANY	123	248,234	9,250	3.7	14,675	5.9	21	15
HONG KONG	55	762,971	23,079	3.0	60,673	8.0	14	13
SOLOMON ISLANDS	23	26,662	2,208	8.3	1,255	4.7	11	3
FIJI	9	25,708	1,169	4.5	1,398	5.4	5	2
HAWAII	8	1,706	106	6.2	117	6.9	1	3
KENYA	3	4,148	60	1.4	378	9.1	3	1
SURINAME	2	571	21	3.7	141	24.7	0	1

Table 3.2: Summary statistics by supplier for ornamental finfish. See Table 3.1 for explanations of the columns. The row labels are specific to this table and do not correspond with Table 2.4.

Supplier	Consignments	Total	Deaths (%)	DOA (%)	Importers	Countries
a	249	5,341,248	1.99	4.0	20	9
b	236	1,824,320	0.87	4.9	5	5
c	205	1,512,062	2.15	4.7	17	8
d	186	2,447,230	0.86	2.1	15	8
e	164	2,109,435	1.31	3.2	5	7
f	164	1,320,189	2.54	2.6	10	7
g	146	4,072,093	0.81	3.6	1	2
h	125	166,869	8.83	4.9	11	8
i	123	1,286,828	0.26	3.8	1	3
j	121	183,226	7.59	6.5	18	9
k	113	216,233	12.38	7.1	19	10
l	102	1,369,029	0.43	5.2	2	3
m	101	769,035	0.93	2.5	8	9
n	94	821,884	3.30	7.3	24	9
o	91	160,873	21.11	5.8	15	9
p	86	134,442	9.41	7.3	12	7
q	78	1,052,995	1.52	5.7	1	2
r	75	684,440	0.35	2.6	1	3
s	72	62,515	5.00	6.1	4	1
t	64	1,086,105	0.90	4.3	4	7
u	62	563,399	1.23	10.7	4	1
v	59	544,194	0.78	5.4	4	4
w	56	92,095	14.61	3.7	9	6
x	53	735,102	1.71	7.6	2	1
y	44	48,036	18.60	9.2	11	10
z	43	172,191	1.80	4.0	12	6
A	43	345,792	2.71	5.9	6	4
B	41	11,707	1.99	16.6	2	1
C	39	21,911	3.38	25.1	2	2
D	38	341,201	10.56	5.7	7	8
E	37	263,767	49.32	6.8	8	7
F	36	151,571	28.73	8.9	9	6
G	34	417,590	1.02	1.5	6	5
H	33	83,910	4.51	7.5	7	6
I	32	298,498	1.11	6.8	9	4
J	32	20,032	4.18	9.8	2	1
K	30	161,236	0.97	3.7	7	4
L	30	6,530	1.88	17.8	2	1
M	29	64,676	21.38	10.9	12	6
N	29	19,535	4.06	15.4	11	3
O	28	177,450	9.88	3.7	9	7
P	28	63,043	1.78	13.2	10	5
Q	27	159,462	0.42	1.5	4	3
R	25	155,636	1.67	2.2	3	4

Table 3.3: Summary statistics by importer for ornamental finfish. See Table 3.1 for explanations of the columns. The row labels are specific to this table and do not correspond with Table 2.3.

Importer	Consignments	Total	Deaths (%)	DOA (%)	Suppliers	Countries
a	1195	13,785,874	0.85	4.8	43	10
b	623	8,523,303	1.47	3.1	32	10
c	609	526,242	5.90	6.3	55	10
d	413	2,563,754	5.53	7.4	69	11
e	369	7,281,153	1.43	3.8	32	8
f	364	3,640,890	1.02	2.6	14	8
g	173	1,363,431	2.41	4.7	16	5
h	145	638,113	0.54	4.6	22	8
i	142	709,501	2.07	9.2	4	3
j	90	393,591	1.47	5.4	16	5
k	88	869,200	5.65	4.9	7	7
l	82	49,970	6.94	16.3	9	5
m	75	683,187	4.78	6.6	16	7
n	74	43,271	8.24	13.0	0	6
o	74	664,752	4.61	6.6	17	7
p	70	673,570	46.65	4.4	15	4
q	58	48,500	3.87	23.9	8	7
r	57	220,416	2.25	5.6	16	3
s	48	144,986	1.13	2.0	14	6
t	46	119,091	7.76	4.9	9	5
u	46	322,310	4.84	11.3	16	4
v	45	485,489	0.94	4.9	8	4
w	43	170,677	1.42	5.0	5	4
x	42	37,708	0.94	11.1	2	4
y	42	33,216	1.74	9.1	5	7
z	40	66,549	6.35	13.2	10	3
A	37	66,935	3.81	10.9	10	6
B	36	17,199	4.97	49.6	1	3
C	34	464,336	2.61	4.3	12	3
D	31	182,398	1.93	3.5	8	4
E	31	27,282	3.50	9.2	8	5
F	31	14,340	4.97	7.7	7	3
G	30	112,382	15.62	11.4	10	2
H	28	38,016	10.57	20.0	9	3
I	27	302,028	1.77	4.7	16	6
J	25	134,877	1.66	8.4	6	4
K	25	132,519	0.18	3.8	6	4
L	24	290,170	1.90	4.5	8	5
M	24	12,460	4.94	31.1	21	4
N	24	165,837	1.77	9.6	6	4
O	23	131,897	2.77	22.5	6	3
P	23	41,606	1.59	4.0	9	5
Q	22	50,035	11.32	18.1	7	1
R	22	150,121	3.17	5.2	10	6
S	22	7,871	18.26	12.6	10	5
T	20	20,330	6.47	23.3	0	6

3.2 Phase 2 Inspection Rates

Based on the AIMS data, we computed the expected proportion of consignments and tails to come through the Melbourne regional office (SERO). The data upon which the tail table is based are not very reliable, as, for example, month names have been recorded in place of fish counts in many instances (for internal reference, see, e.g., AIMS entry number AAPFFEGH4). Furthermore, the tail count drops drastically and inexplicably from year to year.

Data quality notwithstanding, we estimate that the Melbourne regional office received approximately 38.5% on average of the consignment count during the years 2008–2012 (Table 3.5).

Table 3.4: Percentage of consignments received at each port by year and overall for 2008–2012 (all species). The *Count* margins report the number of consignments. The *Average* row is the unweighted average of the proportions across the years.

Year	FN QLD	NSW	S QLD	VIC	WA	Count
2008	0.8	35.2	25.2	34.0	4.9	1896
2009	0.6	26.9	29.7	39.8	3.0	1749
2010	0.6	27.0	24.3	43.9	4.2	1725
2011	0.4	32.1	28.3	35.2	3.9	1595
2012	0.6	28.2	26.8	39.3	5.1	727
Average	0.6	29.9	26.9	38.5	4.2	
Count	46	2321	2062	2946	317	7692

Table 3.5: Percentage of tails received at each port by year and overall for 2008–2012 (all species). The *Count* margins report the number of tails in millions. The *Average* row is the unweighted average of the proportions across the years. FN Qld contributes only a trace amount and is therefore not reported.

Year	NSW	S QLD	VIC	WA	Count
2008	20.9	0.6	76.0	2.5	11.34
2009	8.9	1.1	87.4	2.7	10.05
2010	20.2	1.2	78.1	0.5	9.44
2011	20.7	2.4	75.5	1.3	5.28
2012	52.1	22.1	11.7	14.1	0.17
Average	24.6	5.5	65.7	4.2	
Count	6.35	0.46	28.77	0.68	36.29

Therefore to approximate the projected throughput for Victoria, we should expect to provide about 40% of the needed national level of tests.

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Appendix A

Inspection Work Instructions for Phase 1

Appendix A

Instruction & Guideline

**Actions and outcomes for non-compliances
arising from on-arrival inspection of
documents and live ornamental fish
consignments**

Summary of main points

Instructions and Guidelines are nationally endorsed and must be followed by all Department of Agriculture (the Department) employees. This document outlines decisions regarding non-compliance(s) identified with documents (health certification and invoice/packing lists) and on-arrival inspection of consignments of imported live freshwater (goldfish and other freshwater species) and marine ornamental fish.

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2 Introduction

This Instruction and Guideline (I&G) is designed to assist the Department's biosecurity officers to make nationally consistent decisions regarding non-compliance identified with documents (health certification and invoice/packing lists) and on-arrival inspection of consignments of imported live freshwater (goldfish and other freshwater species) and marine ornamental fish. Regular use of this guideline will ensure national consistency for non-compliance actions and outcomes.

3 Scope

This I&G applies to:

- ❖ Live imported ornamental fish listed on the Department's List of live freshwater fish suitable for import and the Department's List of live marine fish suitable for import and which are accompanied by a valid DEPARTMENTAL import permit.

This I&G does not include examining or ordering into quarantine of:

- ❖ Any other species of animal.
- ❖ Fish that have been unlawfully imported.
- ❖ Laboratory fish for research purpose.

4 Definitions

Definitions used in this document.

Term	Definition
CA	Competent Authority. The Department recognises Competent Authorities of exporting countries for the purposes of providing health certification of live ornamental fish exported to Australia.
Invoice /packing list	These two terms are often used interchangeably and as such they are listed as one in this document. Whether the document is called a packing list or invoice, the document which accompanies the consignment is expected to represent the species (species name, number per bag and box) arriving and that document should be endorsed by the competent authority and used for audit purposes. Consignments do not require that a packing list and invoice are endorsed. But one of them must be endorsed and represent the consignment in full.
QAP	Quarantine Approved Premises (QAP). In this reference material the QAP being referenced is a class 7.1 QAP, approved for freshwater and/or marine ornamental fish quarantine.

5 Legislation

Commonwealth quarantine laws are contained in the *Quarantine Act 1908* and subordinate legislation including:

- Quarantine Regulations 2000
- Quarantine Proclamation 1998
- Quarantine (Christmas Island) Proclamation 2004
- Quarantine (Cocos Islands) Proclamation 2004
- Quarantine Service Fees Determination 2005
- Quarantine Service Fees (Australia Post) Determination 2005
- Notice of Declaration of a Special Quarantine Zone.

Legislation related to import of live ornamental fish is contained in sections 16A-D, 44B, 46A, 52, 54, 55A, 68, 70-70D of the *Quarantine Act 1908*.

6 Non-compliance identification, action and outcomes

6.1 Non-compliance classification

This guideline lists three (3) levels of non-compliance with respect to the import permit conditions for live ornamental fish. These may apply to a whole consignment, or part thereof. The levels of non-compliance are:

1. Critical:

A critical non-compliance would represent a breach of the *Quarantine Act 1908*, or a major breach of the responsibilities and/or the requirements stated in the import permit conditions. A critical non-

compliance will almost always result in the consignment, or part thereof being directed for destruction or export, OR being held without inspection, pending amended documents.

2. Major:

A major non-compliance would represent a serious breach of the responsibilities and/or the requirements stated in the import permit conditions.

3. Minor:

A minor non-compliance would represent a less serious breach of the responsibilities and/or the requirements stated in the import permit conditions.

6.2 Action in response to non-compliances

This guideline lists four (4) actions which should apply to consignments, or part thereof, when non-compliance in relation to the import permit conditions for live ornamental fish is identified:

1. Direct for destruction or export:

The consignment, or part thereof, should be directed for destruction, or export at the importer's expense and without recompense.

2. Do not inspect, hold pending amended documents:

The consignment should not be sent to the quarantine approved premises (QAP) without sighting copies of (amended) documents. The consignment may be inspected (and sent to the QAP) following the sighting of electronic copies of amended documents, but it must not be released until originals are received. The consignment may be directed for destruction or export if the amended documents are not received in an appropriate timeframe.

***Note:** The importer has the option of destruction or export if they do not wish to obtain amended documents.*

3. Inspect, hold at QAP pending amended documents:

The consignment should be inspected and sent to the QAP. The consignment must not be released until original amended documents are received. The consignment, or part thereof, may be directed for destruction or export if the original amended documents are not received in an appropriate timeframe.

***Note:** The importer has the option of destruction or export if they do not wish to obtain amended documents.*

4. Inspect, send to QAP:

The consignment should be inspected and sent to the QAP.

6.3 Timeframe for actions

The health certificate and invoice/packing list must be audited prior to the initial inspection of a consignment. When consignments are held at the Department, pending amended documents, before they can be inspected the amended documents (copies) must be provided to the Department in a time frame that is agreed to by the relevant region. Out of business hours inspections are not supported. If copies of amended documents are not provided in the appropriate timeframe for the Department to complete the initial on-arrival inspection, the consignment will be directed for destruction or export at the importer's expense.

Consignments may be inspected and sent to the QAP based on copies. But the consignment, or part thereof, may not be released from quarantine until original amended documents have been received, or scanned copies of the original amended documents have been provided to the Department directly from the Competent Authority. The quarantine period does not recommence at the time the original documents are received. Fish may be released at the cessation of their quarantine period providing the amended documents have been provided, and the quarantine period has been satisfactorily completed.

6.4 Multiple non-compliances arising from consignment specific documents

Where multiple non-compliances arise from the same consignment, the most serious outcome and action should be applied to the consignment, or part thereof.

6.5 Destruction of consignments, or part thereof

For destruction, a Departmental veterinary officer or a suitably qualified person under the supervision of a Departmental veterinary officer must euthanise the fish with an approved method as described in the Euthanasia of ornamental fish document found in the [Department of Agriculture Instructional Material Library](#).

7 Additional information for use during the audit of documentation and on-arrival inspection of consignments.

7.1 Commonly used parasite treatments for gill flukes on goldfish

Formalin / formaldehyde	Sodium chloride (salt)
Praziquantel	Potassium permanganate
Organophosphates*	Malachite green
Copper sulphate	Hydrogen peroxide
Methylene blue	Acriflavine
Praziquantel	Others

Absence of a treatment from this list does not mean it is an ineffective treatment. For treatments not listed above, research should be performed or Animal Import Operations Branch consulted.

Note: *If organophosphates (including trichlorfon) are listed, care should be taken to wear PPE when inspecting the consignment.

7.2 Date of arrival and date of export

The date of arrival must be consistent with date of export and the date of Competent Authority certification must be within 7 days of export date.

7.3 Department of Agriculture import permit number

Check ICON import permits to ensure that there is a valid import permit.

7.4 Country of export

Only Department-approved countries are permitted to export live fish to Australia.

Live freshwater fish may only be imported from the following list of Department-approved countries:

Belgium	China
Federated States of Micronesia	Fiji
French Polynesia	Germany
Hong Kong	Indonesia
Kenya	Malaysia

New Caledonia	New Zealand
Philippines	Saudi Arabia
Senegal	Seychelles
Singapore	Solomon Islands
South Africa	Sri Lanka (excluding <i>Carassius auratus auratus</i> - Goldfish)
Thailand	United States of America (USA)

Live marine fish may only be imported from the following Department-approved countries:

Bahrain	Belgium
China	Federated States of Micronesia
Fiji	French Polynesia
Germany	Hong Kong
Indonesia	Kenya
Malaysia	New Caledonia
New Zealand	Philippines
Saudi Arabia	Senegal
Seychelles	Singapore
Solomon Islands	South Africa
Sri Lanka	Taiwan
Tanzania	Thailand
United States of America (USA)	Vanuatu

7.5 Document requirements

All documentation (health certificates and invoice/packing list) must be audited prior to the inspection of the fish consignment and must meet the Department's Minimum documentary requirements policy. Each part of the health certificate must be completed in full. Every page must be endorsed (signed, dated and stamped) by the Competent Authority and all documents (health certificate and invoice/packing list) pertaining to the consignment must include the necessary evidence that the import conditions have been met. Original documents must accompany the consignment.

The date on the invoice and/or packing list must be within the timeframe of export and arrival of the consignment of the fish. Invoice and/or packing lists that include the scientific name of imported fish without the official stamp cannot be accepted. If the health certificate has been amended, this must be counter-signed, endorsed and dated by the Competent Authority.

The Department will accept:

- Official government stamps located anywhere on the documentation.
- Part of the stamp with endorsement on each page.
- Official government stamp on all pages with signature and date on one page of the documentation as long as there is a link between all documents.

4.5.1 Invoice/packing list

A unique number (invoice number, air waybill number or health certificate number) must be included on the invoice and/or packing list and must be also documented on the health certificate. All pages must be stamped and signed by the Competent Authority.

Note: In the case of single species (e.g. goldfish), so long as the health certificate contains the full scientific name and number of tails, an endorsed invoice/packing list is not required.

4.5.2 Health certificate

Check that each page of the health certificate pertaining to the consignment has been endorsed by the Competent Authority. All pages of the health certificate must be a linked with either the invoice number, air waybill number or health certificate number to ensure that each page of the health certificate pertains to the consignment.

7.6 Acceptable scientific names which can be included on the health certificate and/or invoice/packing list

The scientific name (and number being imported) for each fish species, in each box must be included on the health certificate and/or invoice/packing list. Each species must be a permitted species and the acceptable (as listed below) scientific name should be included on the health certificate and/or invoice/packing list. Accepted synonyms are also permitted to be listed.

Freshwater species names are permitted to be listed as follows;

- Where an entire genus is permitted import the genus-only is permitted to be listed. E.g. *Chanda* spp. or *Chanda* sp.. Full scientific name (genus and species) is also permitted.
- Where a species is permitted import the full scientific name (genus and species) must be listed. E.g. *Poecilia reticulata*.

Marine species names are permitted to be listed as follows;

- Where a family is permitted import the genus name-only is permitted to be listed. E.g. Permitted family = Acanthuridae; *Acanthurus* spp. or *Acanthurus* sp. are permitted to be listed. Full scientific name (genus and species) is also permitted. E.g. *Acanthurus achilles*
- Where an entire genus is permitted import the genus-only is permitted to be listed. E.g. *Alectis* spp. or *Alectis* sp.. Full scientific name (genus and species) is also permitted. E.g. *Alectis ciliaris*.
- Where a species is permitted import the full scientific name (genus and species) must be listed. E.g. *Plotosus lineatus*.

7.7 Guidelines for commonly encountered non-compliances

The following table should be used as a guide to actions and outcomes regarding non-compliances identified during audit of documentation and on-arrival inspection. This guideline should be used in conjunction with the relevant work instruction and the audit forms; *On-arrival inspection and document audit form – goldfish, freshwater and marine fish*. Your supervisor should be informed of any non-compliances and proposed actions. Case-by-case assessment may be required. Contact Animal Import Operations Branch only following use of this guideline and consultation with your supervisor.

Key: QAP = quarantine approved premises, CA = Competent Authority.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
All documentation (invoice/packing list and health certificate)	None of the documentation is certified by the Competent Authority	Non-compliant, action.	Do not inspect, hold pending amended documents.	Critical	Policy and import conditions require that all consignments be accompanied by health certificates and invoices which have been certified by the CA. Without endorsement by the CA the health status of the fish is unknown.
	No documentation arrives with the consignment and copies are not available.	Non-compliant, action.	Do not inspect, hold pending documents.	Critical	Policy and import conditions require that all consignments be accompanied by health certificates and invoices. The consignment cannot be inspected if there are no documents present as it is unknown where the CA has attested to the health status of the fish.
Date of issue of health certificate	Original documentation is absent but copies are available.	Non-compliant, action.	Inspect, hold at QAP pending original documents.	Major	Copies are satisfactory to allow consignment to be sent to the QAP pending original documents being provided. Note: Originals must be provided before the consignment may be released.
	Date of issue of health certificate is >7 days before	Non-compliant, action.	Do not inspect, hold pending amended documents.	Critical	Policy and import conditions require that the fish are inspected within 7 days of export. If the health certificate is signed more than 7 days before export

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
	export.				the fish could not have been inspected less than 7 days before export.
	More tails arrive than is certified on the documents. The number of fish in the total consignment is more than 5% higher than the number of fish certified.	Non-compliant, action.	Inspect, hold at QAP pending amended documents.	Major	If the CA is willing to certify to amended documents then there is no quarantine risk as they are attesting that they have inspected all the fish in the consignment and that they are eligible for export to Australia. Note: If amended documents are not received, all of the fish of the species which is under certified must be directed for destruction or export. This is because there is no way to tell which fish have been certified and which have not.
Tail numbers are inconsistent between the number which arrive and the number certified on the documents.	More tails arrive than is certified on the documents. The number of fish in the total consignment is not more than 5% higher than the number of fish certified.	Non-compliant, no action	Inspect, send to QAP	Minor	Due to the great difficulty in counting live fish, it is common to have minor discrepancies between the count made at the border and the count made by the competent authority at the time of certification. Minor discrepancies between these two counts do not indicate a biosecurity risk or system failure. Seeking amended documentation is not warranted in these situations.
	Less tails arrive than is certified on the documents.	Non-compliant, no action	Inspect, send to QAP.	Minor	No additional risk, CA is certifying based on inspection of the premises (which will likely hold more fish than those being exported to Australia) rather than inspecting individual fish.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
Import permit number	The importer does not have a valid import permit.	Non-compliant, action	Direct for destruction or export.	Critical	The Quarantine Act 1908 requires that importation of live fish (animals) have an import permit which must be valid at the time the goods are imported into Australia.
	Import permit number listed on the health certificate is invalid (or absent, expired) but the importer has a current valid import permit.	Non-compliant, action	Inspect, hold at QAP pending amended documents.	Major	Providing the importer has a valid import permit there is no quarantine risk with allowing the consignment to be held at the QAP pending amended documents.
Invoice / packing list	There is no invoice or packing list included with the consignment.	Non-compliant, action.	Do not inspect, hold pending amended documents.	Critical	Import permit conditions require that each consignment must be accompanied by documents that include the identification number of each box, and the scientific name and number of the contained fish. Note: In the case that a consignment contains only goldfish and the full scientific name is listed on the health certificate, it is appropriately endorsed and the numbers are consistent with the number of tails imported, an amended invoice is not required.
	A permitted species is listed as a non-permitted species.	Non-compliant, action.	Direct for destruction or export.	Critical	No assurance that animals have been certified by the CA if the wrong name is on the invoice and therefore the health status of the fish is unknown.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
	Non-permitted species listed on the invoice.	Non-compliant, action.	Direct for destruction or export.	Critical	Non-permitted species are not permitted import.
	Competent Authority stamp is absent from the invoice AND there is NO linkage between the health certificate and the invoice (e.g. invoice number, air waybill number etc)	Non-compliant, action.	Do not inspect, hold pending amended documents.	Critical	Minimum Documentary Requirements policy requires that all documents are stamped and signed by an official from the CA. If there is no linkage between the invoice and health certificate AND the CA has not been demonstrated to have sighted the invoice (i.e. stamp not present) it is unclear whether the consignment in question is the consignment which the health certificate refers to.
	There is no linkage between the health certificate and the invoice (e.g. invoice number, air waybill number etc) BUT all pages are signed and stamped by the Competent Authority	Non-compliant, action.	Inspect, hold at QAP pending amended documents.	Major	If there is no linkage between the invoice and health certificate it is unclear whether the consignment in question is the consignment which the health certificate refers to, however if the CA has stamped and signed the invoice there is evidence that the CA has sighted all the documents and the consignment may be sent to the QAP pending amended documents.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
	Competent Authority stamp or signature are absent from the invoice AND there is linkage between the health certificate and the invoice (e.g. invoice number, air waybill number etc)	Non-compliant, action.	Inspect, hold at QAP pending amended documents.	Major	Minimum Documentary Requirements policy requires that all documents are stamped and signed by an official from the CA. Linkage between the invoice and health certificate is sufficient evidence that the CA is attesting to the health of the consignment in question, and therefore the consignment may be to be sent to the QAP pending amended (stamped/signed) documents.
	A permitted species is included in the shipment but not listed on the invoice	Non-compliant, action.	Direct for destruction or export.	Critical	No assurance that animals have been certified by the CA if the wrong name is on the invoice and therefore the health status of the fish is unknown.
	Full scientific name of species not on invoice (except for those where Genera-only suffice).	Non-compliant, action.	Inspect, hold at QAP pending amended documents.	Minor	So long as species can be confidently identified as a permitted species and the incorrect name is a misspelling of the correct name, or a partial scientific name (eg C. auratus), it is okay to inspect and hold at QAP pending amended documents.
Health certificate (freshwater, marine or goldfish)	Health certificate absent.	Non-compliant, action.	Do not inspect, hold pending amended documents.	Critical	Every consignment must be accompanied by a consignment specific health certificate. Consignment is not permitted entry without a valid health certificate.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
	Country of origin (source) health certificate absent (in case where country of origin and country of export are not the same country)	Non-compliant, action.	Inspect, hold at QAP pending amended documents.	Major	If fish are sourced from another country within 6months of export they must have a health certificate from the source country in addition to the country of export. Note: If the health certificate is not obtained from the CA of the country of origin, the fish must be directed for destruction or export.
	Parasite treatment unacceptable or absent on goldfish specific health certificates.	Non-compliant, action.	Do not inspect, hold pending amended documents.	Major	The intent of this risk management measure is to manage the risk of flukes offshore. The fish are not permitted entry if the CA will not attest to them being treated with a parasiticide.
	Dosage rate of parasiticide is absent, but an effective parasite treatment is listed on goldfish specific health certificates.	Non-compliant, action.	Inspect, hold at QAP pending amended documents	Major	If an effective parasite treatment is listed and all other aspects of the inspection are satisfactory, the goldfish may be inspected and sent to the QAP pending amended documents which include the dosage rate.
	Health certificate not signed by Competent Authority official but stamped by Competent Authority.	Non-compliant, action.	Inspect, hold at QAP pending amended documents.	Major	Minimum Documentary Requirements policy requires signature, date, and stamp. As the documents have been stamped with the CA stamp it is known the documents have been sighted by the CA and the consignment may be inspected pending amended documents.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
	Health certificate not stamped by Competent Authority.	Non-compliant, action.	Do not inspect, hold pending amended documents.	Major	Minimum Documentary Requirements policy requires signature, date, and stamp. No CA stamp means it is unknown whether a CA official was the person who signed the health certificate. Therefore consignment cannot be inspected until amended documents are obtained.
	Attestations not present on health certificate.	Non-compliant, action.	Do not inspect, hold pending amended documents.	Major	Policy and import permit conditions require specific attestations on the health certificate. If they are not on the health certificate, the consignment cannot be considered to meet the required health status.
	Exporter name absent	Non-compliant, action.	Inspect, hold at QAP pending amended documents.	Major	The policy and import permit conditions require that the export premise is approved by the CA. If the premise name is not on the health certificate it is unknown if the CA knows which premise the consignment was from and whether they consider it an approved premise. Address is required as part of import permit conditions.
	Exporter contact details absent (phone number, email address etc)	Non-compliant, no action.	Inspect, send to QAP.	Minor	No quarantine risk, only a business risk if importer is unable to contact the exporter for amended documents.
	Contact details of endorsing officer absent (some, or all).	Non-compliant, no action.	Inspect, send to QAP.	Minor	Contact details not required in Minimum Documentary Requirements policy. Phone, fax and email details are only recommended by the OIE Aquatic Animal Health Code Chapter 5.10 Model Health Certificates for International Trade in Live Aquatic Animals and products of Aquatic Animal Origin to be included on health certificates.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
On-arrival inspection of consignment	Non-permitted species (including hybrids and cross-species)	Non-compliant, action.	Direct for destruction or export.	Critical	The policy and import permit conditions require that only fish listed on the Permitted Species List are permitted entry to Australia. Department of the Environment does not consider hybrids and cross-species to be permitted species.
	Mixed species in bag(s) – Very low number of mixed species (1 or 2 for instance) and both species permitted	Non-compliant, action.	Inspect, send to QAP. The labelled fish may be transferred to a tank. The additional species must be destroyed and retained in the quarantine freezer for verification.	Major	The policy and import permit conditions require that each bag must contain only one species. This is a Department of the Environment requirement to aid in species identification.
	Mixed species in bag(s) – High numbers of mixed species and/or non-permitted species	Non-compliant, action.	Direct for destruction or export.	Critical	The policy and import permit conditions require that each bag must contain only one species. This is a Department of the Environment requirement to aid in species identification.
	Invertebrate animals are present in bag(s)	Non-compliant, action.	Direct for destruction or export.	Critical	The policy and import permit conditions require that consignments of live ornamental fish do not contain any prohibited material or material of quarantine concern. Invertebrate animals are not permitted import into Australia.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
	Live coral present in bag (s).	Non-compliant, action.	Direct for destruction or export.	Critical	The policy and import permit conditions require that consignments of live ornamental fish do not contain any prohibited material or material of quarantine concern. Live coral is not permitted import into Australia. Coral may also be subject to legislation under the Convention for International Trades in Endangered Species (CITES).
	Snail(s) present in bag(s).	Non-compliant, action.	Inspect, send to QAP. The snail must be destroyed at the QAP and retained in the quarantine freezer for verification.	Major	The policy and import permit conditions require that consignments of live ornamental fish do not contain any prohibited material or material of quarantine concern. Snails (marine, freshwater or terrestrial) are permitted import to Australia only for human consumption and with varying requirements for processing dependent upon the species. Snails as contaminants in bags of live ornamental fish are not permitted import.
	Clinical signs of pest(s) and/or disease are present in bag(s).	Non-compliant, action.	<p>If:</p> <ul style="list-style-type: none"> fish appear to be diseased, then direct for destruction or export. fish appear to be suffering from travel stress, then inspect, send to QAP. Dead fish must be retained in the quarantine freezer for verification 	Critical	The policy and import permit conditions require that all fish are inspected on arrival and found to be healthy. Some species tend not to travel well and often appear to be suffering travel stress on arrival. Travel stress is not a biosecurity risk and fish should not be destroyed on account of this alone. However, note that travel stress could mask the appearance of other diseases, so when unsure about cause, fish that appear unwell should be directed for export or destruction.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
			<ul style="list-style-type: none"> • unsure, then direct for destruction or export. 		
	Aquatic plants/leaves present in bag(s).	Non-compliant, action.	Direct for destruction or export.	Critical	The policy and import permit state that unapproved plant material (only dried terrestrial plants are permitted) is not permitted import.
	Other material of quarantine concern present in bag(s)	Non-compliant, action.	Direct for destruction or export.	Critical	The policy and import permit state that the bags must not contain any extraneous matter. Note: Vomit is not considered extraneous material providing you are confident it is regurgitated material from the fish.
	Bag(s) do not allow for Departmental biosecurity officers to inspect the fish.	Non-compliant, action.	Ask importer (or their representative) to aid the inspection. If the importer is unable to facilitate inspection; Direct for destruction or export.	Critical	The policy and import permit conditions require that the bags be packed in manner that will facilitate inspection. This includes stocking density, bag weight, bag size, bag colour etc. Note: Bags must not be opened.
	Fish do not meet the species specific size and/or sex restrictions.	Non-compliant, action.	Direct for destruction or export.	Critical	The policy and import permit conditions require that each consignment must include fish that are sufficiently mature to permit accurate identification. Where fish species have minimum size and/or sex requirements they may only be imported if they meet these requirements as outlined by Department of the Environment.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
	Minimum length applies to a species and fry/larvae are present in bag(s)	Non-compliant, action.	Direct for destruction or export.	Critical	Where fish species have minimum size requirements they may only be imported if the average size is equal to, or greater than the size restriction. Fry/larvae will not meet this size restriction and therefore the bag(s) is not permitted import.
	No minimum length applies to a species and fry/larvae are present in bag(s)	Non-compliant, action required if the fry-/larvae cannot be identified as the same species in the bag(s).	Direct for destruction or export.	Critical	The policy and import permit conditions require that each consignment must include fish that are sufficiently mature to permit accurate identification. Where the fry/larvae cannot be identified as the same species in the bag(s), they are not permitted import. If you are confident the fry/larvae are the same species that are in the bag, they may be sent to the QAP. Note: issue for live bearers such as mollies, guppies, platies and also mouth brooders such as pipefish.
	Eggs are present in bag(s)	Non-compliant, action.	Direct for destruction or export.	Critical	Fish eggs (other than those for human consumption) are not permitted import into Australia.
	Suspected genetically modified fish species are present in the bag(s)	Inspect, send to QAP.	Notify importer that they are suspected genetically modified fish and notify Office of the Gene Technology Regulator.	Major	Import permit states that it is the importer's responsibility to identify and ensure compliance with all requirements of any other regulatory and advisory bodies prior to and after importation. Genetically modified fish are not permitted import into Australia as they are subject to regulation under the Commonwealth Gene Technology Act 2000 and Gene Technology Regulations 2011, and corresponding State and Territory legislation.

Document/Consignment	Issue	Action	Outcome	Non-compliance	Justification
	Inert material present in bag(s).	No action.	Inspect, send to QAP.	Permitted	The policy and import permit conditions state that inert extraneous material such as carbon, zeolite, shredded plastic are permitted import so long as the bags can be properly inspected. Material must be disinfected or destroyed at the QAP.
	Vomit present in bag(s)	No action required provided you are confident it is regurgitated material from the fish.	Inspect, send to QAP.	Permitted	Provided you are confident the material has been regurgitated from the fish it should be treated as other waste material from quarantine fish. Request the importer treat the vomit as quarantine waste and disposed of accordingly.
	Dried terrestrial plant present in bag(s).	Plant pathologist must confirm that the leaves are of terrestrial origin.	Inspect, send to QAP.	Permitted	The policy and import permit state that dried terrestrial plants are permitted import. Material must be disinfected or destroyed at the QAP. Note: Bags must not be opened.

8 Work health and safety

This I&G requires Departmental biosecurity officers to examine live fish. Some fish species can cause injury due to sharp spines and care must be taken. Live fish are inspected at Air Cargo Terminals or at the Department's regional office facilities and Departmental biosecurity officers must adhere to all WH&S procedures at these facilities.

It is a requirement that Departmental biosecurity officers performing examinations under this I&G have had Departmental induction training that includes WH&S awareness. Waterproof gloves must be worn and protective apron or coat or sleeve protectors should be worn during inspection of live fish.

Refer to Health and Safety portal for details of The Department's WH&S policy and incident reporting tools.

9 Roles and responsibilities

Authorised Departmental biosecurity officers are responsible for:

- examining documentation to determine if the level of risk is acceptable or unacceptable
- examining the fish to determine if the level of risk is acceptable or unacceptable
- seeking technical advice from the Animal Import Operations Branch as required
- ordering animals into quarantine
- issuing quarantine directions
- notifying importers or agents of decisions/options.

Animal Import Operations Branch is responsible for:

- understanding the animal health risks associated with fish
- assessing and issuing import permits
- providing technical animal health advice to other Departmental officers as required
- issuing industry notices as required
- maintaining ICON
- updating regional Departmental biosecurity officers on new information as required.

10 Record keeping

Copies of all documents associated with, and generated during, the on-arrival inspection process must be kept at the Department's regional office as per Departmental record keeping requirements. Ensure you note all outcomes of on-arrival and document inspection on the Audit forms – *On-arrival inspection and document audit forms – goldfish, freshwater or marine fish*.

11 Related material

This I&G refers to the following materials which are available from the [Department of Agriculture Instructional Material Library](#). It is the responsibility of the Departmental biosecurity officer to ensure they are accessing the most up to date information:

- Standard Operating Procedure - *Quarantine management of imported goods*
- Reference material - *Euthanasia of ornamental fish*
- Instruction & Guideline - *Treatment and testing of live ornamental fish in post-entry quarantine at class 7.1 quarantine approved premises*
- Instruction & Guideline - *On-arrival inspection of live ornamental fish being directed to class 7.1 quarantine approved premises*
- Guideline material: *Fish taxonomy- a guide to understanding the permitted species list*
- Audit forms – *On-arrival inspection and document audit forms – goldfish, freshwater or marine fish*.

The following additional materials may be found on the Department’s website and ICON and it is the responsibility of Departmental biosecurity officers to ensure they are accessing the most up to date information:

- [ICON](#) - *Import conditions for live ornamental freshwater or marine fish*
- [Quarantine Approved Premises Criteria](#) – *Class 7.1 for live freshwater and marine fish*
- [Documentary Requirements](#) - *Minimum documentary requirements policy.*

12 Detailed version history

Version Number	Version Date	Amendment Details
1.0	IMU to complete	Developed to ensure national consistency in actions/outcomes for non-compliances.

Appendix B

Laboratory Work Instructions for Phase 1

Appendix B

Instruction & Guideline

Surveillance testing of live ornamental fish - - Trial One – Melbourne

Summary of main points

Instructions and Guidelines are nationally endorsed and must be followed by all Department of Agriculture (the Department) employees. However, Trial One is a short term trial to be held the Department's South East Region (SER) Melbourne office facilities and as a result this document will not be endorsed nationally. This document outlines decisions surrounding the surveillance testing of live ornamental fish at the SER office facility during on-arrival quarantine inspections.

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2 Introduction

This Instruction & Guideline (I&G) is designed to assist the Department's biosecurity officers (officer) to make decisions surrounding the surveillance testing of live ornamental fish at on-arrival quarantine inspection.

3 Scope

The activity in this I&G are to test and validate operational procedures when live ornamental fish are selected for surveillance testing in the following circumstances:

- ❖ During on –arrival quarantine inspection at the Department's SER office facilities, an officer will undertake fish inspections as per the current *On-arrival inspection of live ornamental fish being directed to a class 7.1 quarantine approved premises* and identify non-compliances using the current *Actions and outcomes for non-compliances arising from on-arrival inspection of documents and ornamental fish consignments*.
- ❖ In addition, this I&G *Surveillance testing of live ornamental fish - Trial One – Melbourne*, is specific to Trial One and the related surveillance activities. These activities will occur concurrently and does not remove any requirements of other instructional material.

4 Definitions

Definitions used in this document.

Term	Definition
Surveillance testing	The periodic on-arrival screening of imported ornamental fish for a specific disease or for biological markers of disease.
<i>Aeromonas salmonicida</i>	A gram-negative bacillus that is critical to both wild and cultivated fish.
Spring viraemia of carp virus (SVCV)	SVCV is caused by a bullet shaped Rhabdovirus. It is also known as <i>Rhabdovirus carpio</i> and it causes an acute, systemic, contagious disease. The virus causes haemorrhage and inflammation of the swim bladder leading to abdominal distension, lethargy, imbalance, swimming on their side and sinking. Affected fish may also appear pale, with darkening of the skin and fins +/- pin point haemorrhages.
Iridovirus (Megalocytivirus)	Megalocytivirus. Viruses within this genus are causative agents of severe disease accompanied by high mortality in multiple species of marine and freshwater fish. Following infection fish became lethargic and exhibited severe anaemia, petechiae of the gills, and enlargement of the spleen caused by enlarged basophilic cells within infected organs.

5 Legislation

Commonwealth quarantine laws are contained in the *Quarantine Act 1908* and subordinate legislation including:

- Quarantine Regulations 2000
- Quarantine Proclamation 1998
- Quarantine (Christmas Island) Proclamation 2004
- Quarantine (Cocos Islands) Proclamation 2004
- Quarantine Service Fees Determination 2005
- Quarantine Service Fees (Australia Post) Determination 2005
- Notice of Declaration of a Special Quarantine Zone.

Legislation related to import of live ornamental fish is contained in sections 16A-D, 44B, 46A, 52, 54, 55A, 68, 70-70D of the *Quarantine Act 1908*.

6 Surveillance testing - Trial One

The surveillance testing trial will gather baseline information on all consignment of ornamental fish arriving at the Department's SER office facility.

For the duration of this Trial One the ornamental fish used will come from bags of non-compliant fish that have been nominated for destruction by the importer.

6.1 Collection of consignment data

The collection of consignment, health and sampling data is a key component of the Trial One. For every consignment inspected during the trial, complete the following documents/forms as applicable:

- *On-arrival inspection and document audit forms.*
- *Invoice/packing list* – if the invoice/packing list does not identify the number of fish in each bag (e.g. total number per box is listed), calculate and write the number of tails per bag on the packing list. If marine fish are packed in >1 fish per bag also make note on the packing list/invoice.
- *Ornamental Fish – Data Sheet A* (Appendix 1) – this should be completed for any bags of fish which have minor non-conformities or low levels of mortality/morbidity but which are still sent to the QAP.
- *Ornamental Fish – Data Sheet B (Signs of Ill Health in Bags)* (Appendix 2) – this should be completed for bags of fish listed on Data Sheet A (where applicable).
- *PDI interception summary sheet* (PDI form) (Appendix 3) – one form should be completed for every bag of fish which are sampled during the trial.
- *Ornamental Fish – Data Sheet C (Sampled Fish)* (Appendix 5) – this should be completed for all bags of fish which are taken for sampling (and which will therefore have a PDI number).
- *Sample Tracking Spreadsheet* – should be completed as soon as samples are taken and updated as necessary.
- *AFDL Specimen Advice Note* (AAHL laboratory submission form) (Appendix 6) should be completed for each bag of fish which are sampled and sent to AAHL.
- Complete the *OSP Movement of Quarantine Material* register when the samples have been sent to AAHL.

The original of the data sheets should be kept with the consignment paperwork and a copy of all documents emailed to the Department and CEBRA on a weekly basis.

6.1.1 List of documents to be completed for each consignment

Compliant consignment where all bags are directed to the QAP

- Invoice/Packing list
- *On-arrival inspection and document audit forms*
- Data Sheet A (if applicable)
- Data Sheet B (where applicable)

Consignments with major non-compliance resulting in direction for export or destruction of bag(s)

- Invoice/Packing list
- *On-arrival inspection and document audit forms*
- *PDI interception summary sheet*
- Data Sheet A (if applicable)
- Data Sheet B (where applicable)
- Data Sheet C
- *AFDL Specimen Advice Note* (AAHL laboratory submission form)

- *Sample Tracking Spreadsheet*
- *OSP Movement register*

6.2 Identification of bags of fish for sampling

Fish to be sampled during this trial must come from bags of fish with a major non-compliance, as determined using *Actions and outcomes for non-compliances arising from on-arrival inspection of documents and ornamental fish consignments*, identified during the on-arrival inspection and which the importer has been given the option to export or destroy. Only those fish that the importer has nominated for destruction may be used.

For each non-compliant bag of fish the officer will:

- Set aside the bag of fish for testing until inspection of consignment is complete.
- Complete a PDI interception summary sheet (PDI form). See example of completed PDI form in (Appendix 3). There should be a separate Incident for each bag of non-compliant fish from the consignment.
- Once inspection and PDI form is completed, notify appropriate officer in Operational Service Program (OSP). Do not euthanize the fish until having liaised with OSP.

6.3 Entering details into Incidents Client Database

The officer or a person authorised on their behalf, enters details of the fish to be sampled into Incidents Client Database. See example of *Trial One specific details to enter into appropriate fields* (Appendix 4). Once specific details have been entered into Incident Client Database, notify appropriate officer in OSP, and give them the PDI Interception Summary Sheet which will have the relevant Bottle ID Number on it.

6.4 Recording in AIMS

The officer or a person authorised on their behalf, access the AIMS database and locate the AIMS entry that is subject of the incident to be recorded. While in the AIMS database, enter incidents details:

- Select the line for the entry the incident relates to. Make sure both line tabs are selected.
- Either press the New Incident button; or select Create Incident from the Line menu.
- For entering incidents details see instructional material, Reference – *Creating an Incident*.

6.5 Euthanasia of fish

Those bags of non-compliant fish that the importer (or their agent) chooses to destroy should be placed in a container (un-opened) until the on-arrival inspection is finalised. The officers should inform OSP at the end of the on-arrival inspection that there are fish to be sampled. The officers should not euthanize the fish until they have consulted with OSP and it has been agreed that OSP can immediately process the fish. If fish are ready for sampling after 3.30pm, inform OSP of their presence, but do not open the bags. OSP may choose to hold the bag overnight and process in the morning. If there are non-compliant goldfish on a Friday, animals cannot be sampled. In this case, non-complaint goldfish should be destroyed as per standard conditions. For animal welfare reasons, do not hold these bags over the weekend.

Use a disposable plastic cup to capture the fish and place in a container of appropriate water. A disposable plastic cup can only be used to sample fish from one non-compliant bag. It cannot be reused and must be appropriately disposed of. Euthanise the non-compliant fish as per the reference material *Euthanasia of ornamental fish*. Identify which ornamental fish risk groups the bag of non-compliant fish belongs to in order to confirm the sample method and specific tests the fish will require (see table 1).

Sample each bag of non-compliant fish. In the initial stages of the trial, OSP and the fish officers may choose to sample more than the minimum number of fish required for each risk group in order to practice their techniques. This is acceptable and the samples should be handled appropriately and sent for testing.

6.6 Selection of individual fish for sampling

6.6.1 Poeciliids, gouramis and cichlids

Select two fish for sampling, preferably the two weakest/unhealthiest looking fish. Where there are no weak or unhealthy looking fish, take any two fish. Do not select a fish that was dead prior to inspection. Fish that were seen to die during inspection can be selected.

6.6.2 Goldfish

Select three fish per bag for sampling, preferably the three weakest/unhealthiest looking fish. Where there are no weak or unhealthy looking fish, take any two fish. Do not select a fish that was dead prior to inspection. Fish that were seen to die during inspection can be selected.

6.6.1 Other freshwater fish species

Select one fish per bag, preferably the weakest/unhealthiest looking fish. Where there are no weak or unhealthy looking fish, take any fish. Do not select a fish that was dead prior to inspection. Fish that were seen to die during inspection can be selected.

6.6.2 Marine fish

As marine fish are packed for export with one fish per bag, the one fish will be sampled. Do not select a fish that was dead prior to inspection. Fish that were seen to die during inspection can be selected.

Note: Where a bag of non-compliant fish are all dead before inspection commenced, they are excluded from sampling and must be disposed of appropriately.

6.7 Processing fish for testing

6.7.1 Sample identification and numbering

Each fish species within a consignment which is being sampled should be given a new PDI number. Each individual fish should then also be given a separate number with regards to the sample preparation and labelling. For example, if a consignment has goldfish and gouramis being taken for sampling, that consignment will have x2 PDI numbers (bottle ID number). For the goldfish there will be the PDI bottle ID number plus 'a', 'b' and 'c' labels, for the gouramis there be a PDI bottle ID number plus 'a' and 'b'.

Example:

Goldfish: <i>Aeromonas salmonicida</i> sample	– 203861a
Goldfish: Spring Viraemia of Carp Virus sample	– 203861b
Goldfish: histology sample	– 203861c

These numbers should be written on the sample containers (where applicable – if fresh fish write on the outside of the bag). The sample identification numbers along with other relevant information should be inputted in the *Sampling tracking spreadsheet*. The PDI bottle ID number should be written on the *AFDL Specimen Advice Note* (AAHL Submission Form) in the “*Submitter Reference Number (optional)*” section for reconciliation of testing results from AAHL with the samples taken. Ensure the specific test and sample type (i.e. whole fish for *Aeromonas salmonicida* testing) is written on the AAHL Submission Form in the “*Examination Requested*” section. Ensure the “*Submitter*” details are filled in appropriately and that the “*Reason for Submission*” section lists “The Department of Agriculture Ornamental fish trial”.

6.7.2 Preparation of fish for specific tests

OSP will process the fish for testing; however OSP may also transfer these activities to officers with appropriate skills.

Details on sampling methods for the specific pathogens and the risk groups of fish are below and in table 1.

The materials and equipment used for processing and testing include:

- 80% ethanol fixing solution.
- 10% formalin fixing solution (to be supplied by AAHL).
- Hanks buffered salt solution transport media (to be supplied by AAHL).
- AQUI-S solution for euthanasia.
- Sampling equipment including disposable plastic cups, disposable gloves, new sample bags, new rubber bands to seal bags, new sample containers.
- Preparation equipment including disposable scalpels, tweezers, pins for pinning fish to tray during dissection, Styrofoam trays and aluminium foil.
- Ethanol for cleaning
- Paper towel
- Parafilm for sealing transport tubes/jars.
- Zip lock or heat sealed bags.
- Ice
- Eskies
- Large containers to transport sample bottles in.
- Buckets/containers to hold fish

Note: Officers with appropriate skills can process the fish for testing under OSP guidance.



Table 1: Brief guide to processing non-compliant fish for Trial 1.

Fish group	Fish size	Total fish required to be sampled per bag	Specific test	Sample type	Brief guide to processing samples (see individual sections for full instructions regarding processing of fish for each specific test)
Goldfish	< 6 cm	3	<i>Aeromonas salmonicida</i>	Whole fresh fish	Pat dry, bag, put on wet ice. Transport immediately.
			SVCV	Whole fresh fish	Pat dry, bag, put on wet ice. Transport immediately.
			Histology	Tail off and slit open along body cavity	Place in tube containing 10% formalin.
	> 6 cm	3	<i>Aeromonas salmonicida</i>	Whole fresh fish	Pat dry, bag, put on wet ice. Transport immediately.

			SVCV	Dissected organs (including gill)	Place in 5ml tube containing Hanks Buffered Salt Solution transport media (red). Transport immediately.
			Histology	Dissected organs (including gill)	Place in tube containing 10% formalin
Poeciliids, Gouramis and Cichlids	< 6cm	2	Iridovirus (megalocytivirus)	Tail off and slit open along body cavity	Place in tube containing 80% ethanol.
			Histology	Tail off and slit open along body cavity	Place in tube containing 10% formalin
	> 6 cm	2	Iridovirus (megalocytivirus)	Dissected organs (including gill)	Place in tube containing 80% ethanol.
			Histology	Dissected organs (including gill)	Place in tube containing 10% formalin

Fish group	Fish size	Total fish required to be sampled per bag	Specific test	Sample type	Brief guide to processing samples (see individual sections for full instructions regarding processing of fish for each specific test)
Other freshwater fish	< 6cm	1	Histology	Tail off and slit open along body cavity	Place in tube containing 10% formalin
	> 6 cm	1	Histology	Dissected organs (including gill)	Place in tube containing 10% formalin
Marine fish	< 6cm	1	Histology	Tail off and slit open along body cavity	Place in tube containing 10% formalin
	> 6 cm	1	Histology	Dissected organs (including gill)	Place in tube containing 10% formalin

Note: When processing the fish be sure to keep the area as clean as possible. Do not use the same items between fish without flaming in alcohol first. Also be aware of not using dissection tools on the “outside” and “inside” of the fish without flaming with alcohol between. Be sure to cool the tools after flaming and before using again.

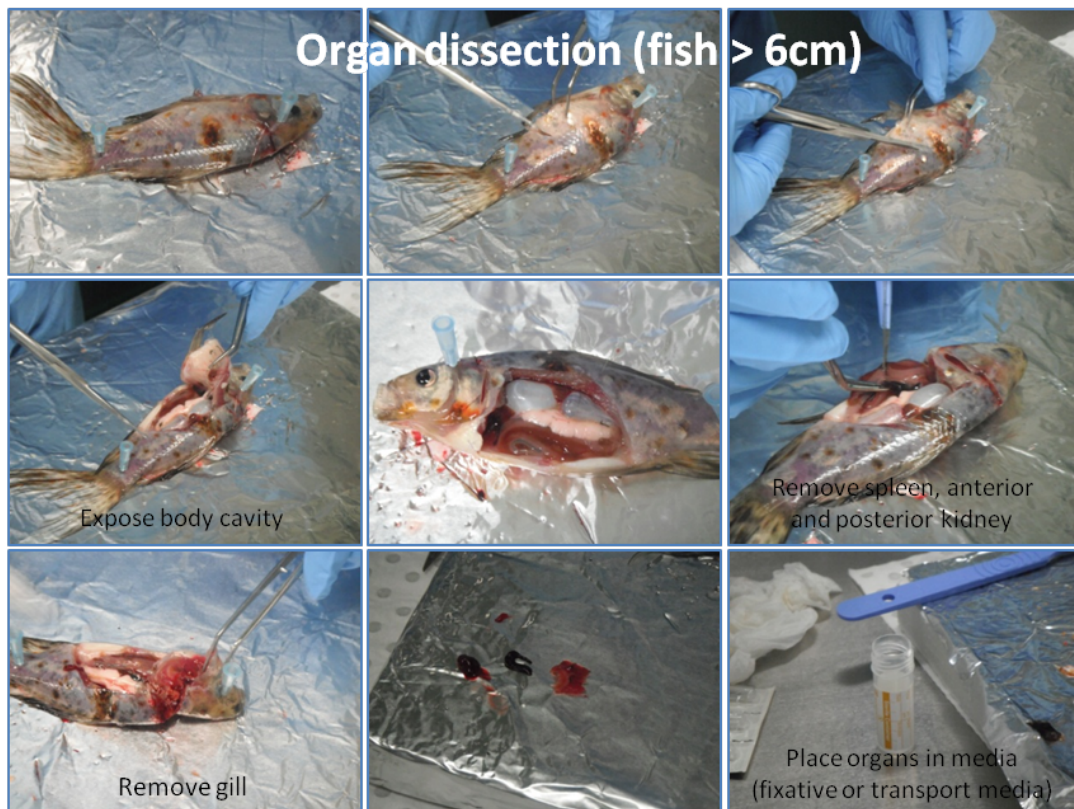
6.7.2.1 Sample preparation for *Aeromonas salmonicida* testing

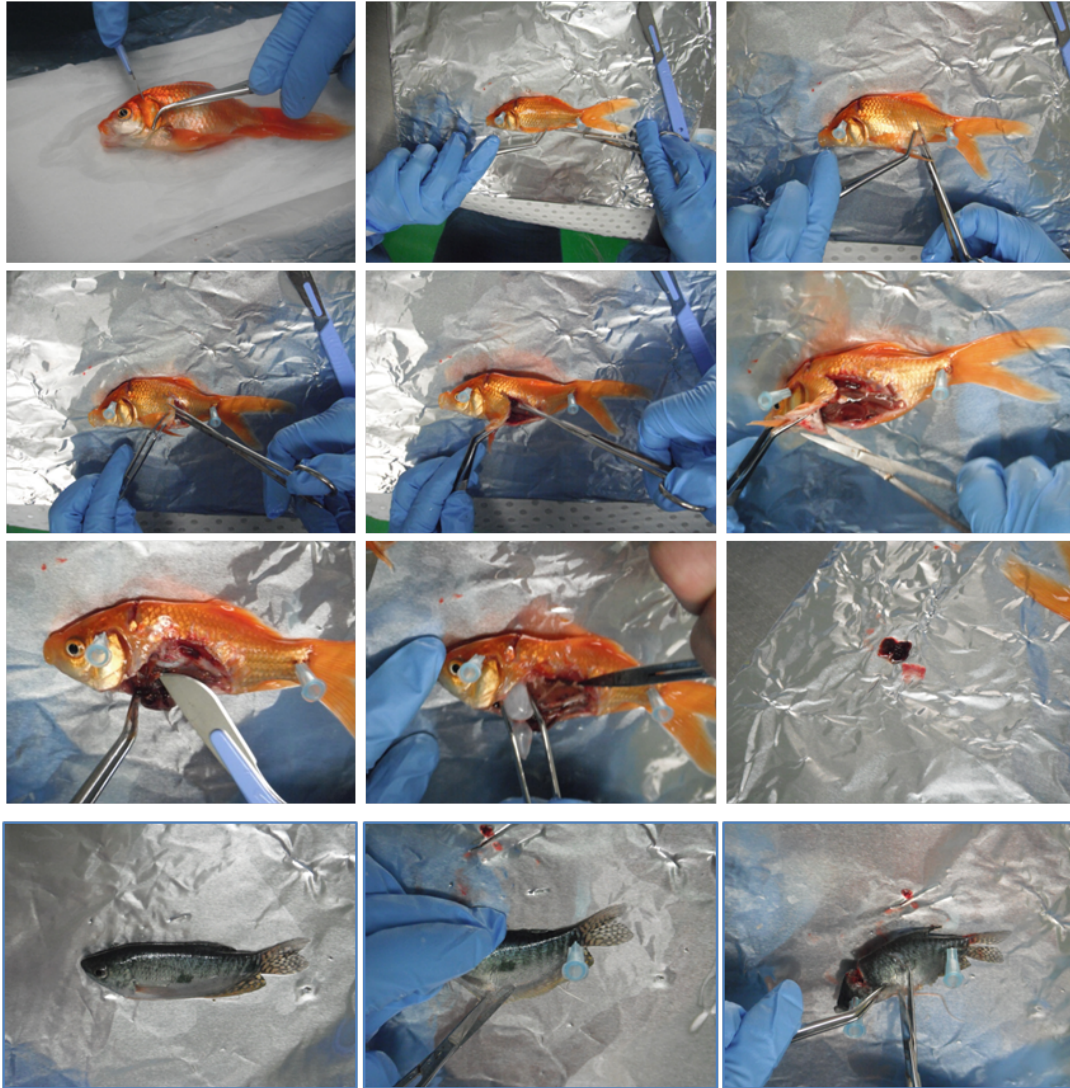
Whole, euthanised fish can be submitted to the testing laboratory and should be transported at 4°C on wet ice. Once the fish has been euthanised, pat it dry with clean paper towel, place it in a sample bag and then place on wet ice (ice with a small amount of water in it). Ensure the sample bag is labelled with the appropriate PDI number and individual identifying number for the fish.

6.7.2.2 Sample preparation for Spring Viraemia of Carp Virus (SVCV) testing

For fish less than 6 cm, whole, euthanised fish can be submitted to the testing laboratory and should be transported at 4°C on wet ice. Once the fish has been euthanised, pat it dry with clean paper towel, place it in a sample bag and then place on wet ice (ice with a small amount of water in it). Ensure the sample bag is labelled with the appropriate PDI number and individual identifying number for the fish.

For fish > 6 cm, a pool of kidney (anterior and posterior), spleen and gill should be taken from the fish. Use the pins to keep the fish attached to the Styrofoam sampling board. Slice the fish open along the body cavity and remove the flap across the body cavity to expose the organs (see pictures below – *Organ dissection (fish >6cm)*). Once body cavity organs are exposed, use a new scalpel to remove the organs (see picture below). This pool of organs should be put in a tube containing Hanks Buffered Salt Solution transport media (red liquid) with the appropriate PDI number and individual identifying number for the fish. Samples for virus isolation need to be transported in a minimal volume of transportation media. The transportation media will be supplied by AAHL in 5 mL aliquots. These tubes can be stored in the freezer until needed. Defrost before use and once a tube has been opened it must be used or discarded.





6.7.2.3 Sample preparation for Iridovirus testing

For fish less than 6 cm, whole, tail-off euthanised fish can be submitted to the testing laboratory in 80% ethanol. Fish should be euthanised, the tail taken off and the body cavity cut open to allow penetration of the fixative. The fish should be put in a small tube with the 80% ethanol and labelled with the appropriate PDI number and individual identifying number for the fish.

For fish > 6 cm, a pool of kidney (anterior and posterior), spleen and gill should be taken from the fish. Use the pins to keep the fish attached to the Styrofoam sampling board. Slice the fish open along the body cavity and remove the flap across the body cavity to expose the organs. Once body cavity organs are exposed, use a new scalpel to remove the organs (see pictures above – *Organ dissection (fish >6cm)*). This pool of organs should be put in a tube containing 80% ethanol and labelled with the appropriate PDI number and individual identifying number for the fish.

6.7.2.4 Sample preparation for histology

For fish less than 6 cm, whole, tail-off euthanised fish can be submitted to the testing laboratory in 10% formalin. Fish should be euthanised, the tail taken off and the body cavity cut open to allow penetration of the fixative (see photo below – *Sample preparation for histology (fish <6cm)*). The fish should be put in a small tube with the 10% formalin and labelled with the appropriate PDI number and individual identifying number for the fish.



For fish > 6 cm, a pool of kidney (anterior and posterior), spleen and gill should be taken from the fish. Use the pins to keep the fish attached to the Styrofoam sampling board. Slice the fish open along the body cavity and remove the flap across the body cavity to expose the organs. Once body cavity organs are exposed, use a new scalpel to remove the organs (see pictures above – *Organ dissection (fish >6cm)*). This pool of organs should be put in a tube containing 10% formalin and labelled with the appropriate PDI number and individual identifying number for the fish.

6.8 Disposal of waste material

Dispose of all quarantine risk material and disposable equipment in the quarantine waste bins located in the in-house inspection room and/or in appropriate sharps-containers. Fish tissue waste should be stored in a freezer in a bag clearly labelled as “Quarantine waste”. The bag should be disposed of in the quarantine waste bins immediately prior to their emptying. This will prevent any odours occurring. In addition, clean all surfaces and reusable equipment used in sampling and preparation with either ethanol or other effective disinfectant at the appropriate rate.

6.9 Transport processed fish to laboratory

The samples must be transported to the laboratory as soon as possible by overnight courier. AAHL recommend using *Geelong Document & Parcel Express Pty Ltd*. The courier will need to send their invoices directly to the Department.

It is SER’s responsibility to liaise with the courier to ensure the samples will arrive at AAHL in time for them to be processed. Take into account the time that the on-arrival inspection will take, the time for processing at SER office, the time that the sample will be delivered to AAHL and AAHL’s requirements for arrival time. Hold the fish over night if necessary and able to.

When considering time frames ensure:

- Fresh tissue/fish must be processed by the laboratory within 24 hrs of the fish being euthanised. This time constraint must be considered against courier times to allow the laboratory to carry out their work against the time limitation. This applies specifically to goldfish samples which must be couriered to AAHL the day the fish is sampled.
- Fixed samples can be stored by OSP and sent on a monthly or volume (when storage is becoming an issue) basis.

Paperwork required to accompany the shipment includes a copy of the Quarantine Entry, PDI form and the *AFDL Specimen Advice Note* (Appendix 6). The sample identification numbers (see section 6.7.1) along with other relevant information should be inputted in the *Sampling tracking spreadsheet*. The PDI bottle ID number should be written on the *AFDL Specimen Advice Note* (AAHL Submission Form) in the “*Submitter Reference Number (optional)*” section for reconciliation of testing results from AAHL with the samples taken. Ensure the specific test and sample type (i.e. whole fish for *Aeromonas salmonicida* testing) is written on the AAHL Submission Form in the “*Examination Requested*” section. Ensure the “*Submitter*” details are filled in appropriately and that the “*Reason for Submission*” section lists “The Department of Agriculture Ornamental fish trial”.

Place paperwork in an envelope taped to the top or side of the consignment or contained in a zip lock bag inside the esky.

When a tissue sample is prepared email a copy of the completed specimen advice to AAHL. The original will still need to accompany the sample; however the email is essential as it alerts them to the fact there is an inbound shipment. If it does not arrive in a timely manner they can then alert the courier and the Department.

Fill in OSP's *Movement of Quarantine Material* spreadsheet and the *Sample Tracking Spreadsheet* (located in the *Surveillance Trial* folder in the *Scientific (OSP-PEPQ)* folder on SER J: drive). The *Sample Tracking Spreadsheet* has drop down menus to allow for quick entry of data. Ensure this is filled-in for every sampled fish.

6.10 Laboratory results

The laboratory test report must include on a single document: a definitive statement regarding whether testing has resulted in positive or negative result. The laboratory should be contacted if the test results are not in this format.

The original test result should be kept with the consignment paperwork and a copy emailed to the Department and CEBRA.

7 Work health and safety

This I&G requires the Department's biosecurity officers to examine live fish. Some fish species can cause injury due to sharp spines and care must be taken. Live fish are inspected at Air Cargo Terminals or at the Department's regional office facilities and the Department's biosecurity officers must adhere to all WH&S procedures at these facilities.

It is a requirement that the Department's biosecurity officers performing examinations under this I&G have had the Department's induction training that includes WH&S awareness. Waterproof gloves must be worn and protective apron or coat or sleeve protectors should be worn during inspection of live fish.

Refer to for details of the Department's WH&S policy and incident reporting tools.

8 Roles and responsibilities

The Department's biosecurity officers are responsible for:

- examining documentation to determine if the level of risk is acceptable or unacceptable
- examining the fish to determine if the level of risk is acceptable or unacceptable
- seeking technical advice from the Animal Import Operations Branch as required
- ordering animals into quarantine
- issuing quarantine directions
- initiate a pest interception form
- notifying importers or agents of decisions
- draw sample for testing
- euthanize fish (OSP)
- complete a PDI form
- initiate incident in Incidents Client database
- update AIMS entry with relevant details and directions
- complete specimen advice (OSP)
- update and complete incident in Incidents Client database (OSP)
- prepare sample (OSP)
- submit sample (OSP)
- record test results in Incidents Client and forward copy to the Department and CEBRA (OSP)
- support officers involved in Trial One (OSP)

Animal Import Operations Branch is responsible for:

- understanding the animal health risks associated with fish
- assessing and issuing import permits
- providing technical animal health advice to other officers as required
- issuing industry notices as required
- maintaining ICON
- updating regional biosecurity officers on new information as required.

Animal Biosecurity Branch is responsible for:

- providing surveillance testing advice to the officers as required
- collating and analysing laboratory results
- list applicable laboratories for testing

9 Record keeping

Copies of all documents associated with, and generated during, the on-arrival inspection process must be kept at the Department’s regional office as per Departmental record keeping requirements. All electronic documents including all emails relating to this trial should be saved in the *Surveillance Trial* folder in the *Scientific (OSP-PEPQ)* folder on SER J: drive.

10 Related material

This I&G refers to the following materials which are available from the Department’s Instructional Material Library. It is the responsibility of the officer to ensure they are accessing the most up to date information:

- Standard Operating Procedure - *Quarantine management of imported goods*
- Reference material - *Euthanasia of ornamental fish*
- Guideline material: *Fish taxonomy- a guide to understanding the permitted species list.*
- Instruction and Guideline - *Post-entry quarantine of live ornamental fish at class 7.1 Quarantine Approved Premises.*
- Instruction and Guideline - *Actions and outcomes for non-compliances arising from on-arrival inspection of documents and ornamental fish consignments.*
- Reference – *Creating and Incident.*

The following associated materials may be found on the Department’s website and ICON and it is the responsibility of the Department’s biosecurity officers to ensure they are accessing the most up to date information:

- ICON - *Import condition for live ornamental freshwater or marine fish*

11 Detailed version history

Version Number	Version Date	Amendment Details
One	05/03/2013	Initial draft
Two	08/04/2013	Include comments on initial draft
Three	10/04/2013	Include outcomes from discussions with the Department of Agriculture Aquatic Animal Program
Four	29/04/2013	Incorporate comments from regional visit

12 Appendices

APPENDIX 1

Ornamental Fish – Data Sheet A

(to be completed for any bags of fish which are sent to the QAP but which have a minor non-compliance (e.g. 2/300 guppies dead))

Quarantine Entry (AIMS)..... Supplier Code..... Supplier Country.....

Inspection Date..... Inspector Name.....

Species (Scientific Name)	No. of Fish	Mortality Rate	Morbidity Rate	Visual Signs of Ill Health? [Blank = No]	
				Yes/No	
		%	%		<i>If Yes -> Also Complete Sheet B</i>
		%	%		<i>If Yes -> Also Complete Sheet B</i>
		%	%		<i>If Yes -> Also Complete Sheet B</i>
		%	%		<i>If Yes -> Also Complete Sheet B</i>
		%	%		<i>If Yes -> Also Complete Sheet B</i>
		%	%		<i>If Yes -> Also Complete Sheet B</i>
		%	%		<i>If Yes -> Also Complete Sheet B</i>
		%	%		<i>If Yes -> Also Complete Sheet B</i>

Each line relates to one bag of fish

Additional Notes.....

APPENDIX 2


Ornamental Fish – Data Sheet B (Signs of Ill Health in Bags)

(to be completed for bags listed on Data Sheet A)

Quarantine Entry (AIMS)

Species	Species	Species
<p>Visual signs of ill health? [Tick all that apply]</p> <p><input type="checkbox"/> Significant mortalities in the bag</p> <p><input type="checkbox"/> Loss of colouring or darkening</p> <p><input type="checkbox"/> Abnormal swimming behaviours, hovering at the surface</p> <p><input type="checkbox"/> Lethargy or hyper/hypo-activity</p> <p><input type="checkbox"/> Fin burn/congestion/erosion</p> <p><input type="checkbox"/> Respiratory activity such as rapid gulping</p> <p><input type="checkbox"/> Loss of balance, swimming on the side</p> <p><input type="checkbox"/> Excess mucous secretion</p> <p><input type="checkbox"/> Abnormal swelling</p> <p><input type="checkbox"/> Bulging eyes</p> <p><input type="checkbox"/> Reddening/haemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes)</p> <p><input type="checkbox"/> Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss</p>	<p>Visual signs of ill health? [Tick all that apply]</p> <p><input type="checkbox"/> Significant mortalities in the bag</p> <p><input type="checkbox"/> Loss of colouring or darkening</p> <p><input type="checkbox"/> Abnormal swimming behaviours, hovering at the surface</p> <p><input type="checkbox"/> Lethargy or hyper/hypo-activity</p> <p><input type="checkbox"/> Fin burn/congestion/erosion</p> <p><input type="checkbox"/> Respiratory activity such as rapid gulping</p> <p><input type="checkbox"/> Loss of balance, swimming on the side</p> <p><input type="checkbox"/> Excess mucous secretion</p> <p><input type="checkbox"/> Abnormal swelling</p> <p><input type="checkbox"/> Bulging eyes</p> <p><input type="checkbox"/> Reddening/haemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes)</p> <p><input type="checkbox"/> Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss</p>	<p>Visual signs of ill health? [Tick all that apply]</p> <p><input type="checkbox"/> Significant mortalities in the bag</p> <p><input type="checkbox"/> Loss of colouring or darkening</p> <p><input type="checkbox"/> Abnormal swimming behaviours, hovering at the surface</p> <p><input type="checkbox"/> Lethargy or hyper/hypo-activity</p> <p><input type="checkbox"/> Fin burn/congestion/erosion</p> <p><input type="checkbox"/> Respiratory activity such as rapid gulping</p> <p><input type="checkbox"/> Loss of balance, swimming on the side</p> <p><input type="checkbox"/> Excess mucous secretion</p> <p><input type="checkbox"/> Abnormal swelling</p> <p><input type="checkbox"/> Bulging eyes</p> <p><input type="checkbox"/> Reddening/haemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes)</p> <p><input type="checkbox"/> Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss</p>
<p>Do you think the ill health was caused by transport stress in this instance? [Circle No or Yes]</p> <p>No Yes</p>	<p>Do you think the ill health was caused by transport stress in this instance? [Circle No or Yes]</p> <p>No Yes</p>	<p>Do you think the ill health was caused by transport stress in this instance? [Circle No or Yes]</p> <p>No Yes</p>

Additional Notes:

AOIS PDI Interception Summary Sheet		 Australian Government Department of Agriculture, Fisheries and Forestry		Bottle ID Number 000203861
Officer's name Mr SMITH	Species LIVE ORNAMENTAL FISH	Import <input checked="" type="checkbox"/>	Export <input type="checkbox"/>	Date 04/04/13
Custodian Entry # A CZ PHM4 N3	Fresh water <input checked="" type="checkbox"/>	Country of origin SINGAPORE	Method FRYING	Date of capture 12/3456/2013
Collected at VIC REGIONAL OFFICE	No. of live exports: <input type="checkbox"/>	Isolation <input checked="" type="checkbox"/>	Condition (head/ventral only) <input checked="" type="checkbox"/> Alive <input type="checkbox"/> Alive and Dead <input type="checkbox"/> Dead <input type="checkbox"/> Unknown	Stage (ventral only) <input type="checkbox"/> Eggs <input type="checkbox"/> Larva <input type="checkbox"/> Pupa <input type="checkbox"/> Nymph <input type="checkbox"/> Adult
No. of live exports: <input type="checkbox"/>	Infection <input type="checkbox"/> Insect <input type="checkbox"/> Seed	Disease <input type="checkbox"/> Infection <input type="checkbox"/> Light <input type="checkbox"/> Moderate <input type="checkbox"/> Heavy	Comments 2X FISH SUBMITTED FOR FURTHER TESTING V125456	

APPENDIX 4

Incident Client (PROD) Version 1.3.0

File Search Help

Search | Most Recently Used | Incident's Detail

New Save Delete Print

Bottle Number: 203861
 Incident Date: 4/04/2013
 Interception Number: []

State: Vic
 Program: Import Clearance
 Reporting Officer: Cliff, Nicole

Interception Where | Interception What | Identification

Import/Export Type: Import Export PEQ

Mode: Sea Air Parcel/Post

Country Of Origin and Notes: Singapore

Transport Method: Air Cargo

Vessel: []

Flight/Voyage Number: []

Port Of Loading: []

Import/Owner: []

Collection Point and Address: ANTINE DESIGNATED PREMISES - CUSTOMS HOUSE, TULLAMARINE - V1023

Detection Point: Export Post Quarantine Detection
 Incurstion Quarantine Intervention Point
 Offshore ICE

Status: Active
 Quarantine Entry: []
 Line: 0

Incident found here: Goods Packing Container/Other

Goods:

Desc	Quantity
Fish Freshwater Live	1

Packing:

Desc	Quantity
[]	1

Container/Other: []

Affected Container(s): External Internal Both None

Container: [] Site Found: []

Bottle Number:

Incident Date:

Interception Number:

State:

Program:

Reporting Officer:

Detection Point

Export Post Quarantine Detection

Incursion Quarantine Intervention Point

Offshore ICE

Status:

Quarantine Entry:

Line:

Interception Where Interception What Identification

Phytosanitary Certificate

Timber Marks

Fumigation Certificate

Yes No Unknown

Type of Pre-Border Treatment

- Autoclave & Steam
- Cold Storage
- Ethylene Dibromide
- Ethylene Oxide
- Formaldehyde, Formagas and Form
- Gamma Irradiation
- Glyphosate
- Heat treatment and Moist Heat treat

Treatment Provider Name and Address

AFAS Treatment Provider Number

ISPM 15

Yes No

Organism

Invertebrate Disease Seed Vertebrate NA

Infestation

Few Moderate Large

Condition

Alive Alive & Dead Dead Unknown

Specimen for Identification

Yes No

Frass

Comments

2 * Live freshwater ornamental fish submitted for further testing - TRAIL STAGE

Biosecurity Incident Category

- Animal Material
- Bark
- Documentation Concerns
- Evidence of plant disease or microorg
- Failed Fumigations DO NOT USE
- Frass/Fresh Borer Holes
- Live Animals USE VERTEBRATE OR
- Mis-Declaration
- Other Invertebrates USE VERTEBRATE
- Plant Material
- Seeds USE PLANT MATERIAL
- Soil/Earth/Sand
- Straw
- Undeclared Item Of Concern
- Vertebrate or Invertebrate
- Water

APPENDIX 5

Ornamental Fish – Data Sheet C (Sampled Fish)

(to be completed for all fish sampled as part of the trial)

Quarantine Entry (AIMS) Code..... PDI Number..... Species.....

Bag number/Identifier	Visual signs of ill health? [Tick all that apply]		
Reason(s) for non-compliance?	Fish number 1	Fish number 2	
<input type="checkbox"/> Fish not certified	<input type="checkbox"/>	<input type="checkbox"/>	Significant mortalities in the bag
<input type="checkbox"/> Mixed species	<input type="checkbox"/>	<input type="checkbox"/>	Loss of colouring or darkening
<input type="checkbox"/> Incorrect species on invoice	<input type="checkbox"/>	<input type="checkbox"/>	Abnormal swimming behaviours, hovering at the surface
<input type="checkbox"/> Non-permitted species	<input type="checkbox"/>	<input type="checkbox"/>	Lethargy or hyper/hypo-activity
<input type="checkbox"/> Unidentified species	<input type="checkbox"/>	<input type="checkbox"/>	Fin burn/congestion/erosion
<input type="checkbox"/> Undersized fish	<input type="checkbox"/>	<input type="checkbox"/>	Respiratory activity such as rapid gulping
<input type="checkbox"/> Wrong sex	<input type="checkbox"/>	<input type="checkbox"/>	Loss of balance, swimming on the side
<input type="checkbox"/> Insect in bag	<input type="checkbox"/>	<input type="checkbox"/>	Excess mucous secretion
<input type="checkbox"/> Snails in bag	<input type="checkbox"/>	<input type="checkbox"/>	Abnormal swelling
<input type="checkbox"/> Eggs in bag	<input type="checkbox"/>	<input type="checkbox"/>	Bulging eyes
<input type="checkbox"/> Plant material in bag	<input type="checkbox"/>	<input type="checkbox"/>	Reddening/hemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes)
<input type="checkbox"/> Coral in bag	<input type="checkbox"/>	<input type="checkbox"/>	Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss
<input type="checkbox"/> Signs of disease	Do you think the ill health was caused by transport stress in this instance? [Circle N or Y]		

AFDL SPECIMEN ADVICE NOTE

CSIRO Animal, Food and Health Sciences (CAFHS)
 Australian Animal Health Laboratory (AAHL)
 AAHL Fish Diseases Laboratory
 Private Bag 24, Geelong, Vic 3220
 Telephone: + 61 3 5227 5000
 Fax: + 61 3 5227 5555
 Email: afdl@csiro.au



AAHL SAN Number (AAHL Use Only)	Page 1 of 1 QA/14.2.9										
<table border="1"> <tr> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> </tr> </table>											

<p><u>Submitter Reference Number (optional):</u></p> <div style="border: 1px solid black; height: 20px; width: 100%;"></div> <p><u>Submitter:</u></p> <p>State/Country:</p> <p>Email: <div style="border: 1px solid black; width: 100%; height: 20px;"></div></p> <p>Telephone (business):</p> <p>Telephone (after hours):</p> <p>FAX:</p> <p><u>Forward account to:</u></p> <p><u>Send additional copies of reports to:</u></p>	<p><u>Submission Category (circle one):</u></p> <p>1 = Routine Submission 2 = Emergency Disease Exclusion (low probability) 3 = Emergency Disease Exclusion (high probability)</p> <p><u>Animal details:</u></p> <p>Species:</p> <p>Date specimens collected:</p> <p>Age/Year class: Farmed <input type="checkbox"/> Wild <input type="checkbox"/></p> <p>Sex:</p> <p>Farm or location:</p> <p>Owner:</p> <p>Origin of population:</p> <p>Population size:</p> <p>Marine zone/Hatchery:</p> <p><u>Water conditions:</u></p> <p>Temperature:</p> <p>Salinity:</p> <p>Other:</p>
<u>Reason for submission:</u>	
<u>Specimen details:</u>	
<u>History and clinical signs:</u>	
<u>Examination requested:</u>	<u>AFDL Use only</u>
<u>Signature of submitter (please also print name):</u>	Date of dispatch to AAHL:
	Date specimens received at AAHL:

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Author	Bernadette O'Keefe	14 th August 2012
Authorised by	Mark Crane	
Published by	Bernadette O'Keefe	

Appendix C

Work Instructions for Phase 2

Instruction & Guideline

Health monitoring of live ornamental fish – Trial 2 – Melbourne

Summary of main points

Instructions and Guidelines (I&G) are nationally endorsed and must be followed by all Department of Agriculture (the Department) employees. However, Trial 2 is a short term trial to be held in the Department's South East Region (SER) Melbourne office facilities and as a result this document will not be endorsed nationally. This document outlines decisions surrounding the health monitoring of live ornamental fish at the SER office facility during on-arrival quarantine inspections.

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2 Introduction

This I&G provided instructions to assist the Department’s biosecurity officers in trialling the proposed ornamental fish health monitoring program. The program has two components:

- A. Sampling, processing and testing of fish from ‘compliant fish bags’, i.e. bags of imported ornamental fish that on inspection are determined not to be associated with significant signs of communicable disease.
- B. Sampling, processing and testing of fish from ‘non-compliant fish bags’, i.e. bags of imported ornamental fish that on inspection are determined to be associated with significant signs of communicable disease.

Each of these components is dealt with separately in this I&G.

3 Scope

The activities in this I&G are to test and validate operational procedures for selecting, sampling and testing live ornamental fish as part of a trial (Trial 2) of a health monitoring system.

The trial is scheduled to be undertaken from May to July 2014.

The sole industry participant is an Importer that has kindly volunteered to take part in this trial. All exporting countries, exporters (suppliers) and all freshwater species imported by the importer are subject to random sampling and testing.

- ❖ During the trial, on-arrival quarantine inspection at the SER office facilities, a departmental officers will continue to undertake fish inspections as per the current *On-arrival inspection of live ornamental fish being directed to a class 7.1 quarantine approved premises* and identify non-compliances using the current *Actions and outcomes for non-compliances arising from on-arrival inspection of documents and ornamental fish consignments*.
- ❖ This I&G *Health monitoring of live ornamental fish - Trial 2 – Melbourne*, is to be applied in addition to the above and does not remove any requirements of the other existing instructional material.
- ❖ The I&G *Health monitoring of live ornamental fish - Trial 2* comprises two components: (A) sampling, processing and testing of fish from ‘compliant fish bags’, and (B) sampling, processing and testing of fish from ‘non-compliant fish bags’ (as described in the introduction section above).

4 Definitions

Table 1 Definitions used in this document.

Term	Definition
Health monitoring	The periodic on-arrival sampling and testing of imported ornamental fish for a specific disease or for biological markers of disease.
<i>Aeromonas salmonicida</i>	<i>Aeromonas salmonicida</i> is a pathogenic bacterium that severely impacts salmonid populations and other species..
Spring viraemia of carp virus (SVCV)	SVCV is a Rhabdovirus also known as <i>Rhabdovirus carpio</i> , capable of causing disease in in several carp species and some other cyprinid and ictalurid fish species.
<i>Megalocytivirus</i> (Iridovirus)	Megalocytiviruses (which include gourami iridovirus) are a sub-group of iridoviruses. Viruses within the <i>Megalocytivirus</i> genus are causative agents of severe disease in multiple species of marine and freshwater fish.

Term	Definition
Compliant/healthy bags	Bags of healthy fish imported by the importer. These bags are selected randomly to test for specific pathogens according to the risk group of fish the species in the bag belongs. The risk groups are fish of the gourami family (subfamilies Luciocephalinae and Macropodusinae of the family Osphronemidae) (hereinafter referred to as gourami/s), cichlids, poeciliids, goldfish and other freshwater species. Information the species that make up each risk group is provided at Appendix 3.
Non-compliant/seized bags	Bags of fish showing significant morbidity/mortality and/or associated with critical non-compliances ¹ imported by <i>any</i> importer. All of these bags are sampled for histopathological examination, as well as specific pathogen testing according to the risk group to which the fish belongs (see Appendix 3). Fish species belonging to the 'other freshwater fish' risk group are only subject to histopathological examination.

5 Legislation

Commonwealth quarantine laws are contained in the *Quarantine Act 1908* and subordinate legislation including:

- Quarantine Regulations 2000
- Quarantine Proclamation 1998
- Quarantine (Christmas Island) Proclamation 2004
- Quarantine (Cocos Islands) Proclamation 2004
- Quarantine Service Fees Determination 2005
- Quarantine Service Fees (Australia Post) Determination 2005
- Notice of Declaration of a Special Quarantine Zone.

Legislation related to import of live ornamental fish is contained in sections 16A-D, 44B, 46A, 52, 54, 55A, 68, 70-70D of the *Quarantine Act 1908*.

6 Health monitoring program – Trial 2: Compliant bags

The health monitoring program trial will

- Develop and test a preliminary communication mechanism to inform South East Region Office (SERO) duty officers on which consignment to sample using a dashboard program attached to the AQIS Import Management System (AIMS). The dashboard program will determine which consignments to sample and the types of testing to which each sampled species will be subject.
- Develop and test a preliminary communication mechanism for SERO duty officers to inform Animal Biosecurity Branch (ABB)/Animal Import Operations Branch (AIOB) of which consignments have been sampled using a dashboard program attached to AIMS.
- Trial and review the sampling, specimen/tissue collection and fixing, storage and transport methods.
- Trial ectoparasitology carried out by Operational Science Program (OSP) staff.
- Trial and assess time, resource and infrastructure/equipment requirements of the health monitoring program at SERO.

¹ As per examples listed in the Instruction & Guideline – Action and outcomes for non-compliances arising from on-arrival inspection of documents and live ornamental fish consignments

6.1 Collection of consignment data

Procedures for on-arrival inspection as per Instruction & Guideline *On-arrival inspection of live ornamental fish being directed to class 7.1 quarantine approved premises* will remain unchanged during the trial - e.g. the importer/broker continues to notify SERO of incoming consignments and the SERO officer checks documentation prior to arrival, etc.

In addition to the normal procedures, the importer will enter consignment information into the Integrated Cargo System (ICS) with each risk group of fish in the consignment identified on a separate line (refer to Section 6.4.2 for each risk group). Through the ICS, the collection of consignment data and random targeting of fish consignments for sampling are automated using a dashboard program added on to AIMS – the Ornamental Fish Trial 2 dashboard. The officer must check the dashboard carefully. The dashboard will show which group of fish in the consignment needs to be sampled and the types of testing that need to be applied (refer to Section 6.4.2 for specific types of testing). The consignment box number to be sampled is selected using the random number generator tool and the associated rules as per Section 6.5.

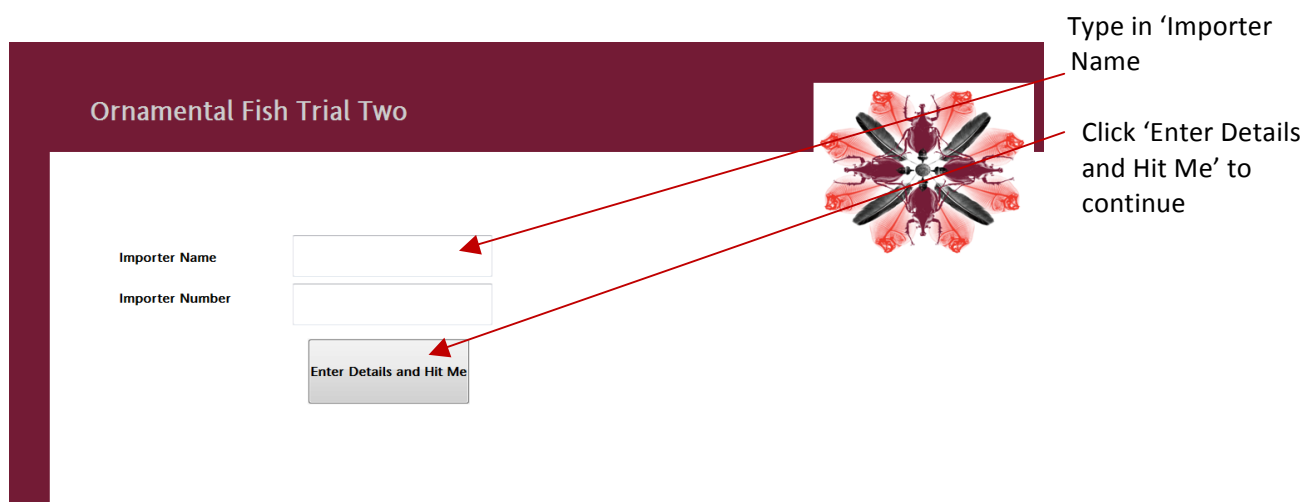
For each box of fish targeted for sampling during the trial, and the bag of fish sampled from the box, the officer must complete the following documents/forms as applicable:

- *On-arrival inspection and document audit forms*
- *fish stats and seizures spread sheet*
- *AFDL Specimen Advice Note* (AAHL laboratory submission form) (Appendix 1) (this should be completed for each bag of fish which are sent to AAHL)
- *DEPI Victoria Request for Laboratory Examination Form* (Appendix 2) (this should be completed for each bag of fish which are sent to the Victorian DEPI Laboratory)

The original of the forms should be kept with the consignment paperwork and a copy of all documents emailed to the Department on a weekly basis.

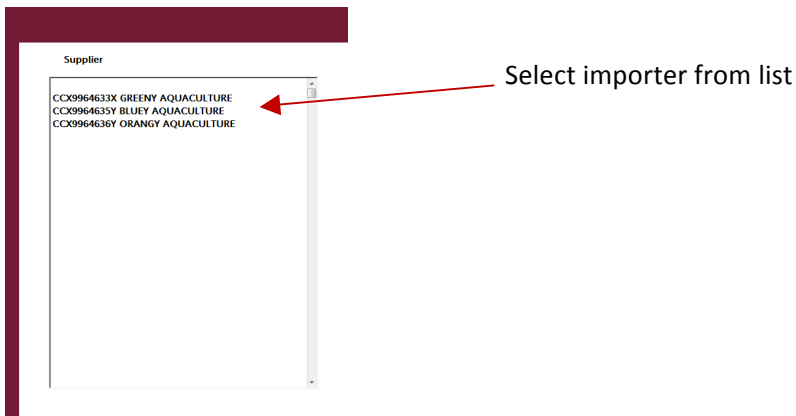
6.2 Check Ornamental Fish Trial 2 Dashboard for compliant/healthy bags

- 1) To access the Ornamental fish Trial 2 dashboard to review testing directions for compliant/healthy bags. After you have entered the Importer name click 'Enter Details and Hit Me'

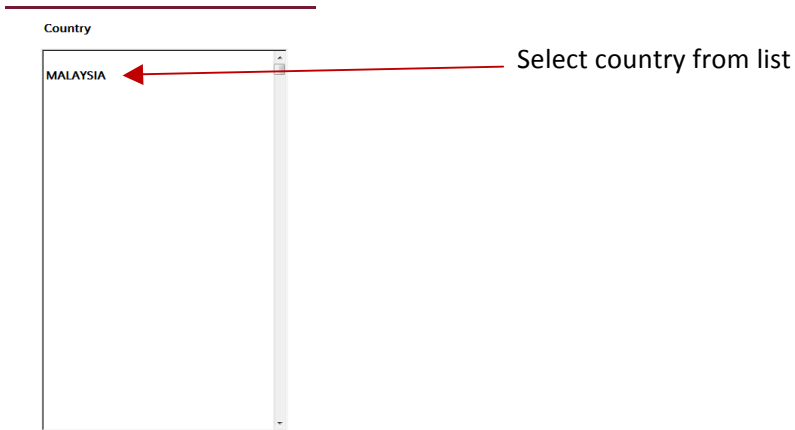


The screenshot shows the 'Ornamental Fish Trial Two' dashboard. It features a dark red header with the title 'Ornamental Fish Trial Two'. Below the header, there are two input fields: 'Importer Name' and 'Importer Number'. A button labeled 'Enter Details and Hit Me' is positioned below these fields. A decorative graphic of a beetle is centered on the right side of the dashboard. Red arrows point from the beetle graphic to the 'Importer Name' field and the 'Enter Details and Hit Me' button. Text annotations on the right side of the image provide instructions: 'Type in 'Importer Name'' and 'Click 'Enter Details and Hit Me' to continue'.

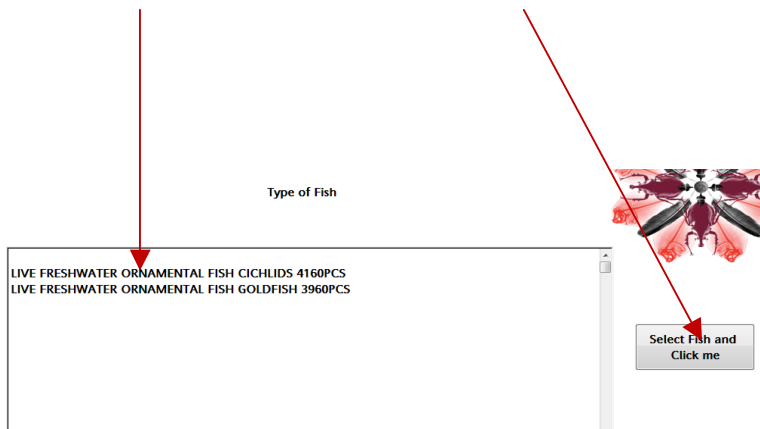
2) Select Supplier of interest from the list.



3) Select Country.

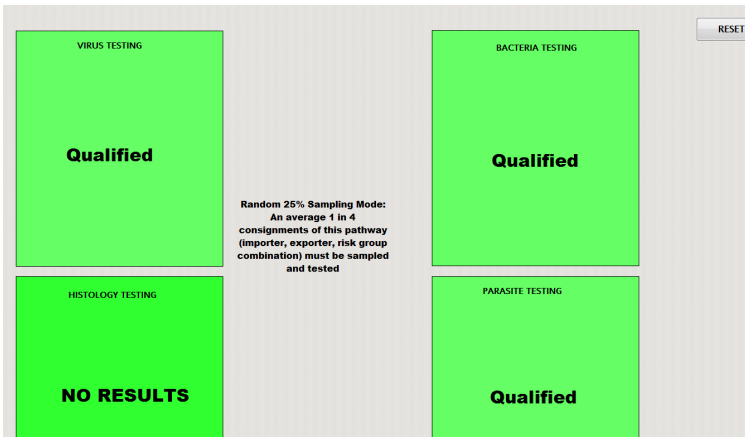


4) Select Type of Fish, and click "Select Fish and Click me".

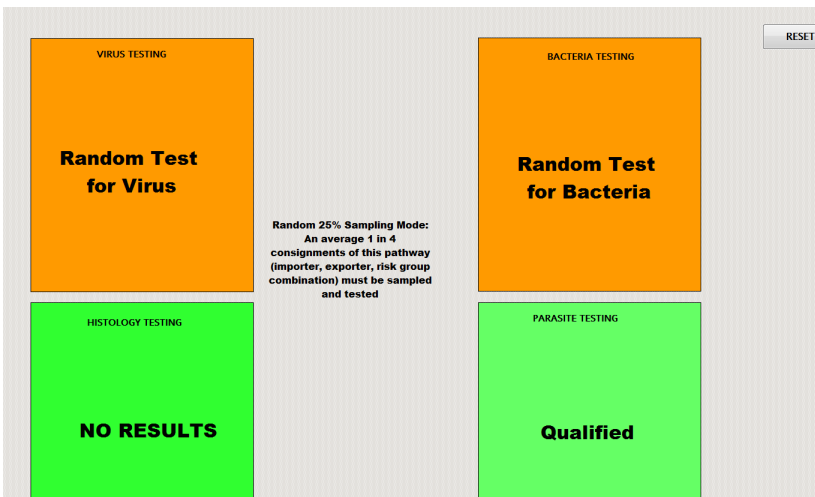


2) Any orange or red tab indicates that this pathway (i.e. importer, exporter, supplier, risk group combination) must be sampled. Any green tab indicates that this pathway is not subject to sampling.

Example 1: The following pathway is not subject to sampling.



Example 2: The following pathway is subject to sampling. As per Section 6.3, assign “Live Fish Virus Testing” and “Live Fish Bacteria Testing” Directions to this pathway (i.e. the Line in AIMS).



Example 3: This pathway is subject to sampling. As per Section 6.3, assign “Live Fish Virus Testing”, “Live Fish Bacteria Testing” and “Live Fish Parasite Testing” Directions to this pathway (i.e. the Line in AIMS).



6.3 Process AIMS entry for compliant/healthy bags

1) To access the AIMS database to enter in and review directions for compliant/healthy bags click on the traffic light symbol (Fig 1).

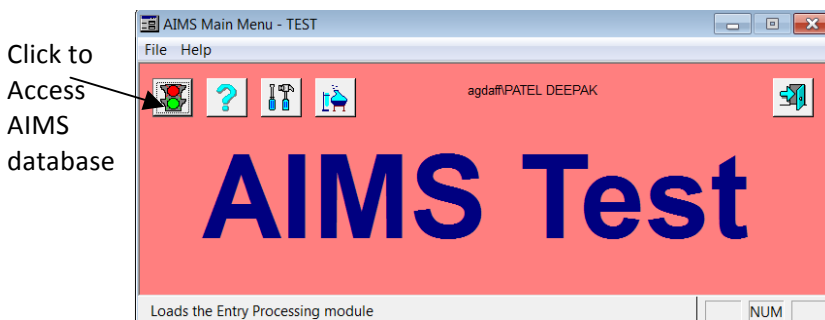


Fig 1: AIMS database start up screen

2) Next, click on the filing cabinet symbol in the top left hand corner of the screen. You will be prompted to enter in the AIMS entry number for the consignment of interest (Fig 2).

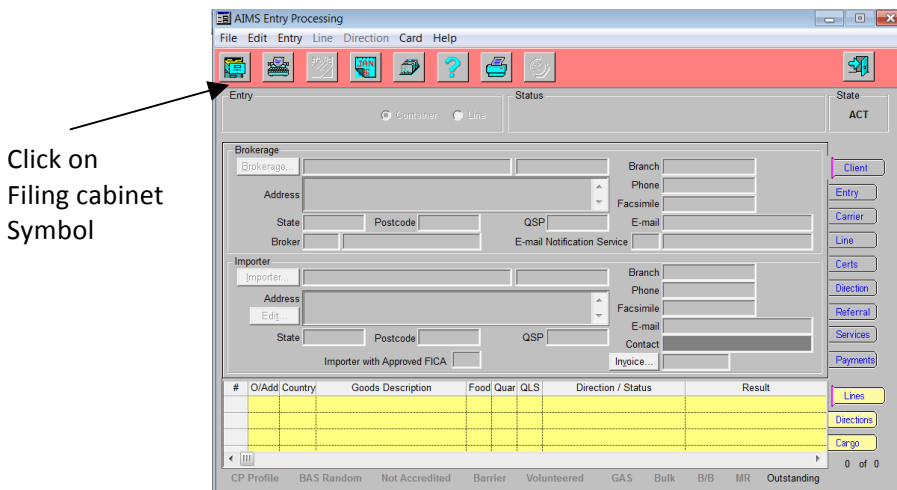


Fig 2: Accessing consignment details screen

3) AIMS will ask if you wish to open the entry even though it is located in another State or Territory. Click on Yes (Fig 3).

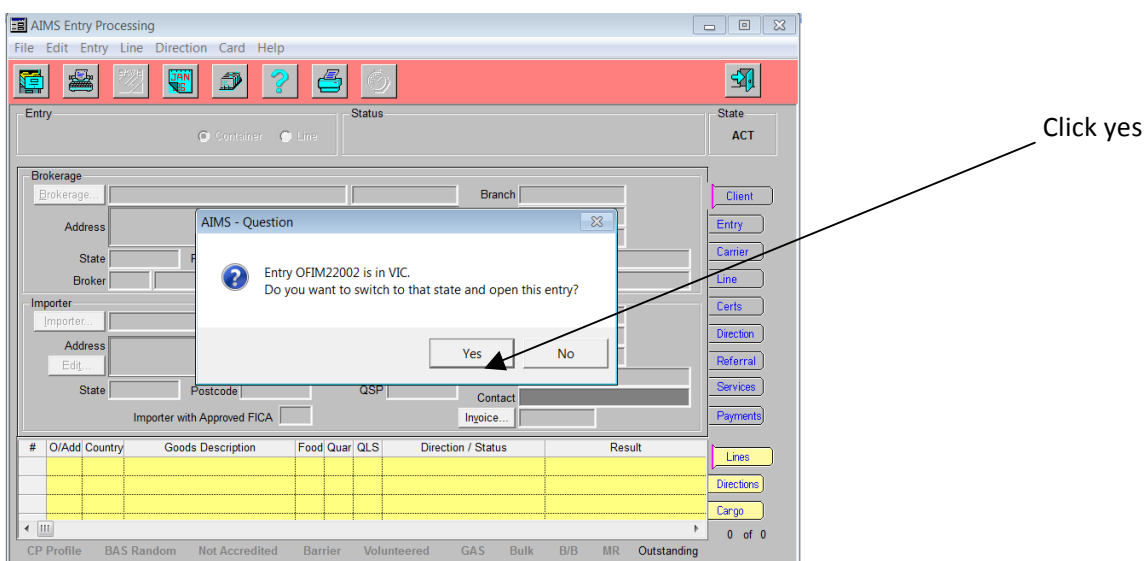


Fig 3: Accessing consignment details screen

4) After clicking yes the database will open at the client tab. This home tab lists all the details about the broker and importer. At the bottom of the screen you will see the Lines for this consignment (Fig 4).

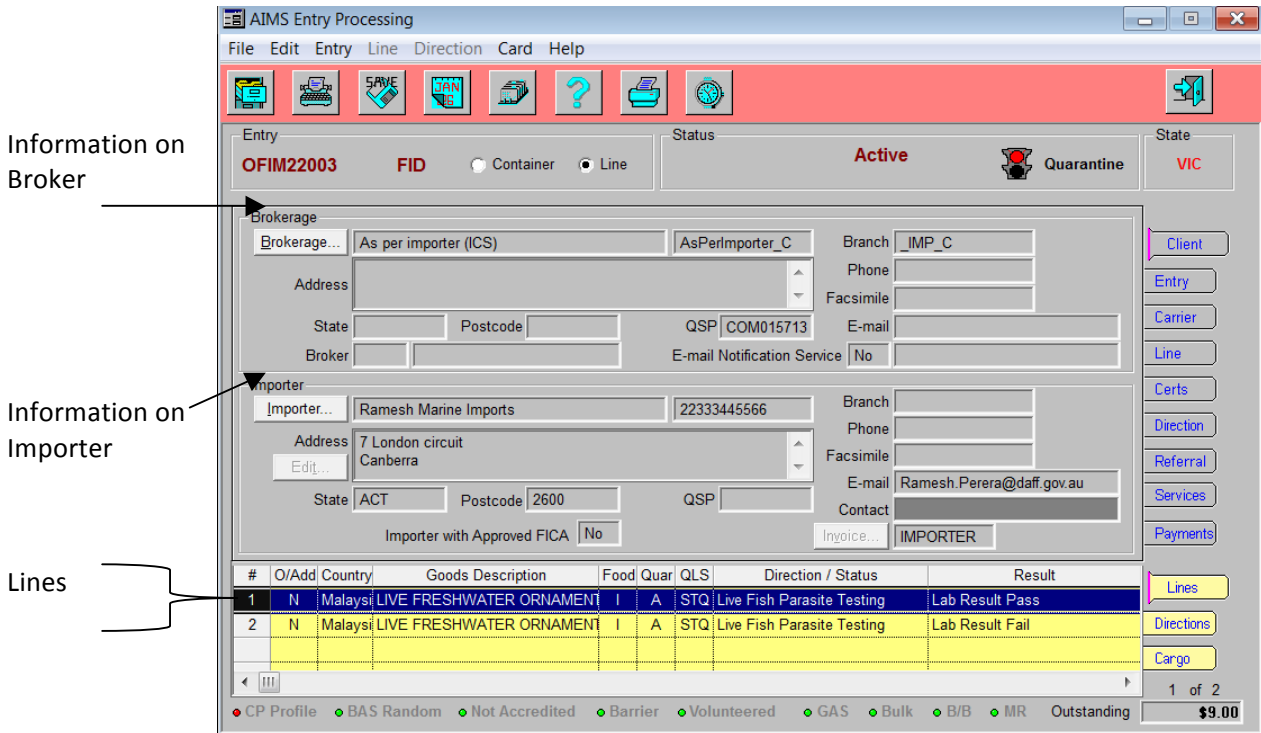


Fig 4: Client page details information about the broker and importer

5) To access the page/tab where you can direct a line/product in for testing, click on the direction tab on the middle right hand side of the screen (Fig 5).

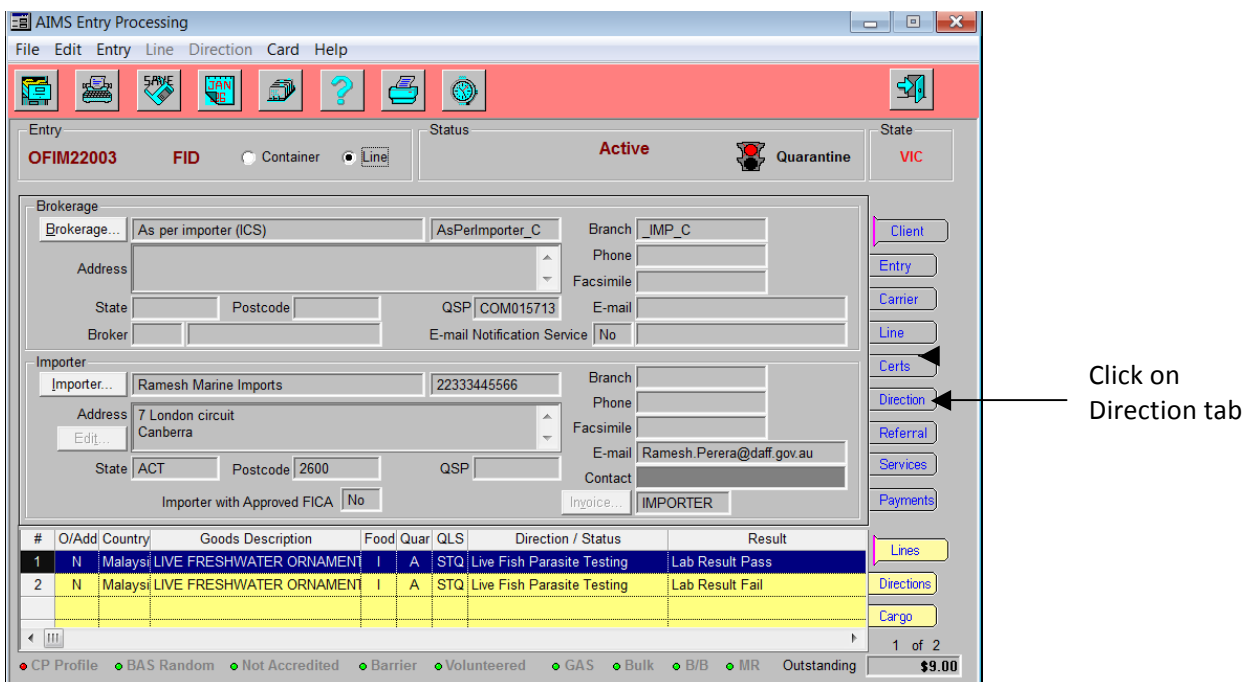


Fig 5: Entering in lines for testing

6) Once in the direction tab, click on Add to enter a line in for testing (Fig6).

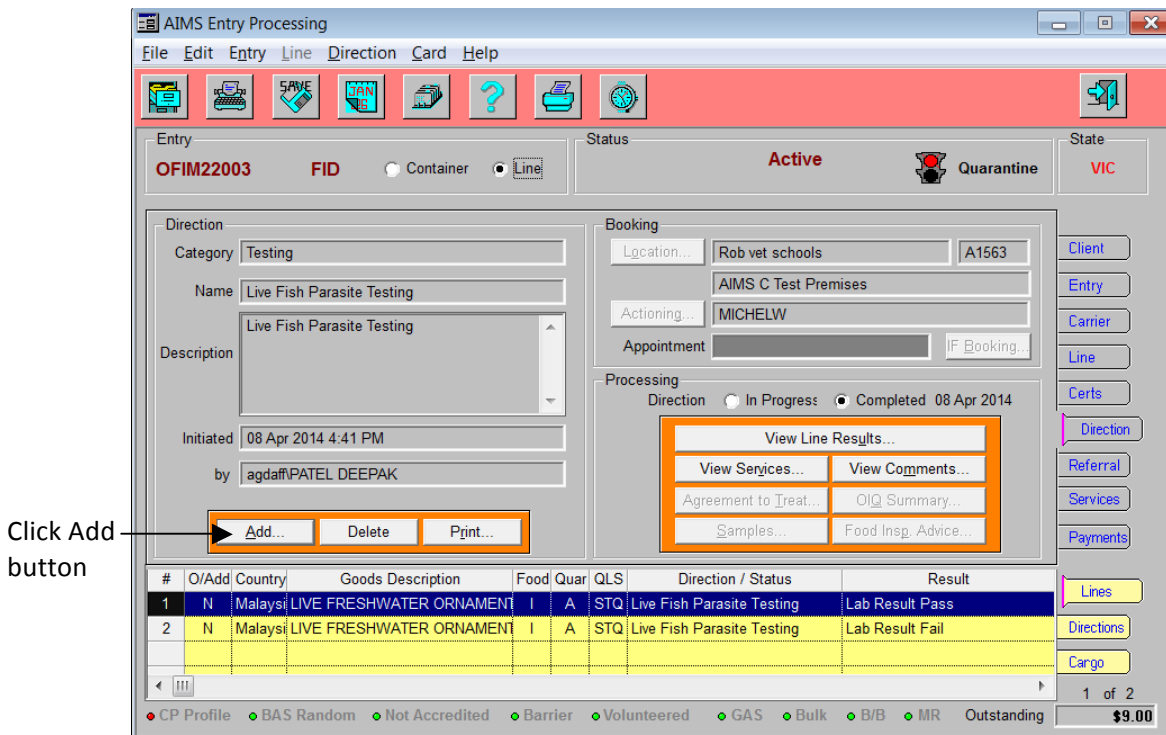


Fig 6) Entering in a line for testing

7) Select which direction you need to apply to a line as per Dashboard instructions on Section 6.2. For example, click on testing in the Direction Category column (left hand side column) and then select “Live Fish Bacteria Testing” from the right hand side column. Click select to confirm selection (Fig 7).

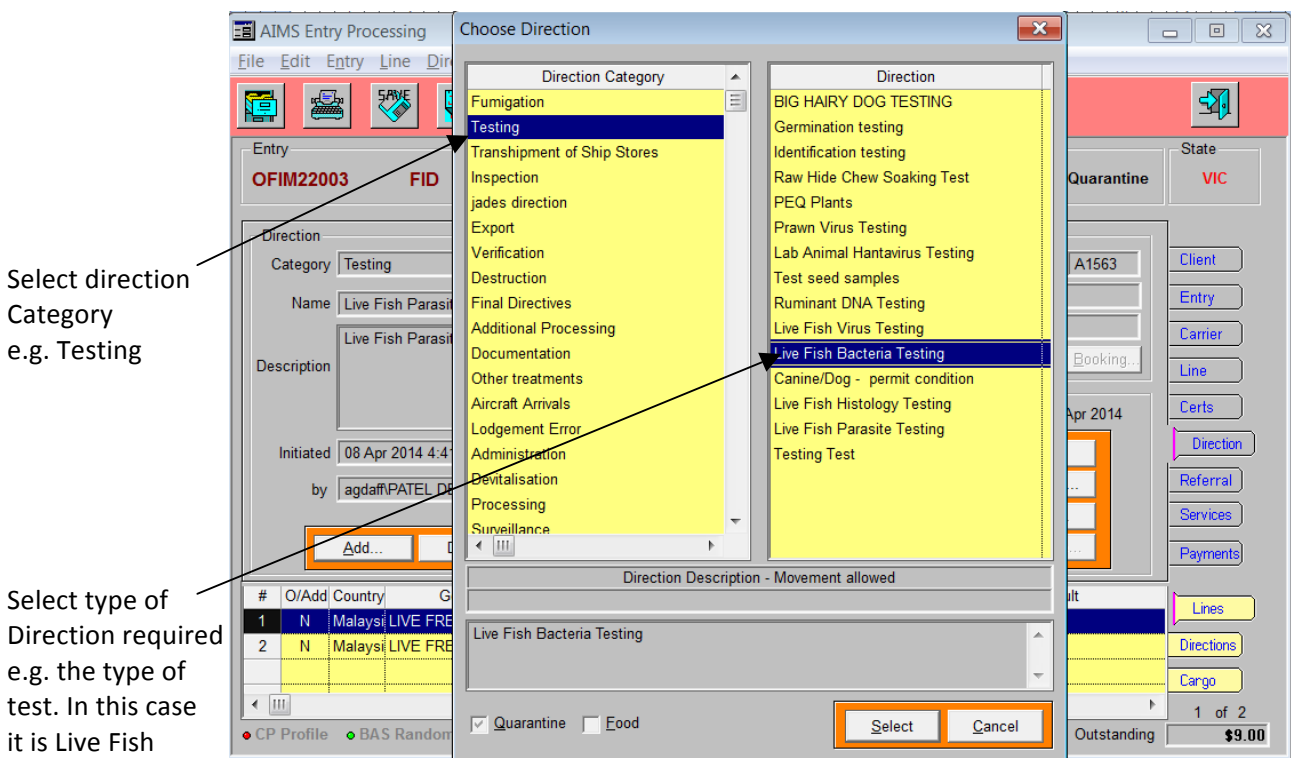


Fig 7: Selecting appropriate Direction

8) You will then be shown a screen (see Fig. 8) which will require you to select the premises of testing. Select the relevant premises from the column on the left hand side and click OK.

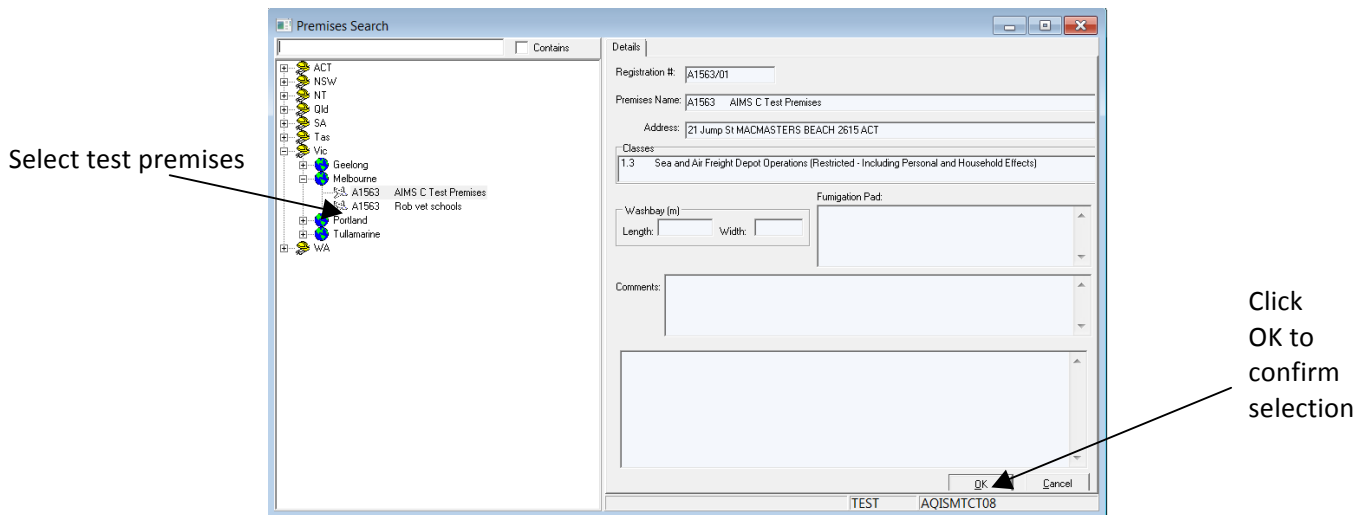


Fig 8: selecting test premises

9) The next screen will prompt you to select which line you would like to enter in for testing. Select a line from the available lines in the box on the left hand side of the screen, then click the Add>> button (Fig 9). You will notice that the line number now appears in the results box in the middle of the screen (see Fig. 10). Click the OK button to submit the line for testing.

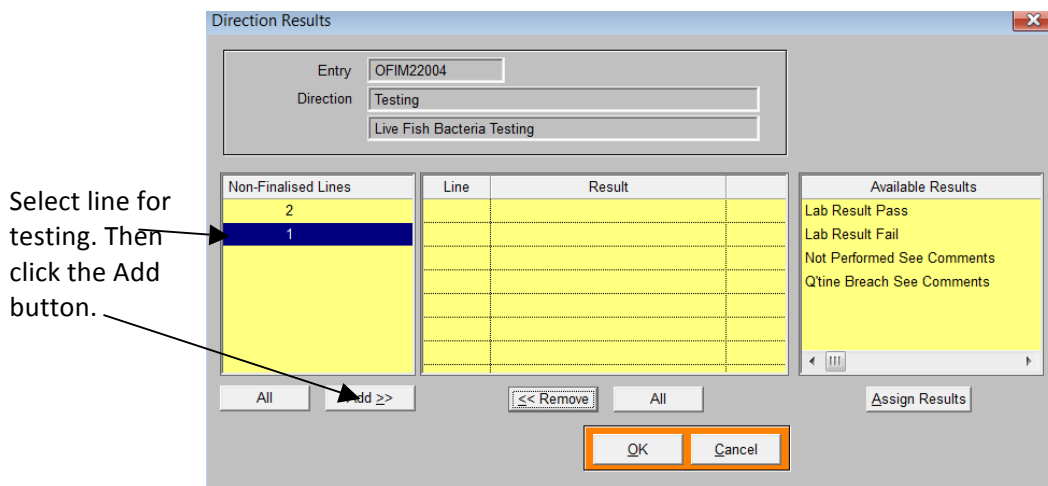


Fig 9: select line for testing

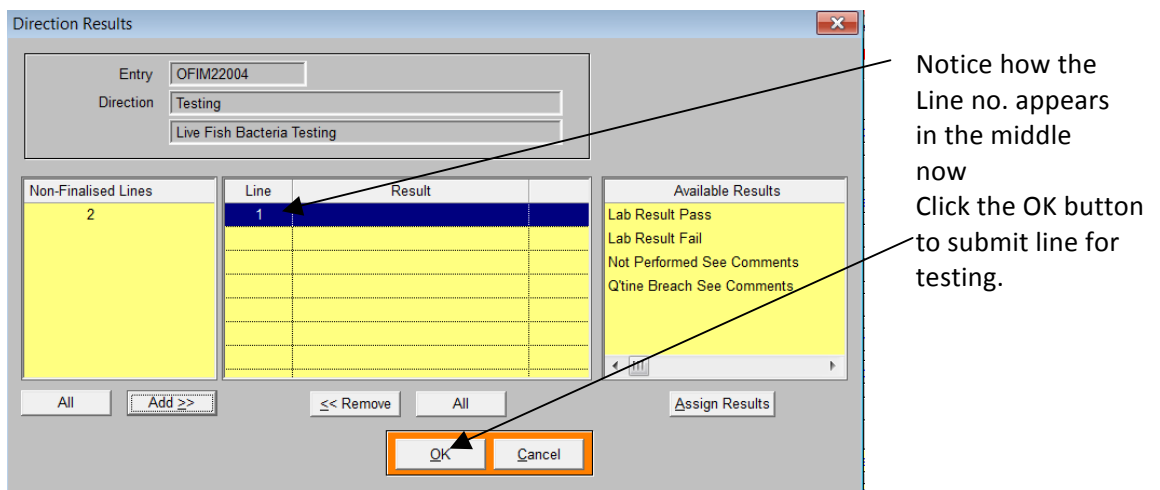


Fig 10: select line for testing

10)

The next step is to proceed with the fish inspection and sampling procedure. Once the results of the testing is made available (this may be several days or weeks later), these test results will need to be entered into AIMS. To do this, go back into the AIMS entry of interest and assign the test result of the line (pass, fail, not performed or quarantine breach). Return to the direction tab and click, highlight the line of interest from the yellow box below by left clicking once and then select 'view line results'.

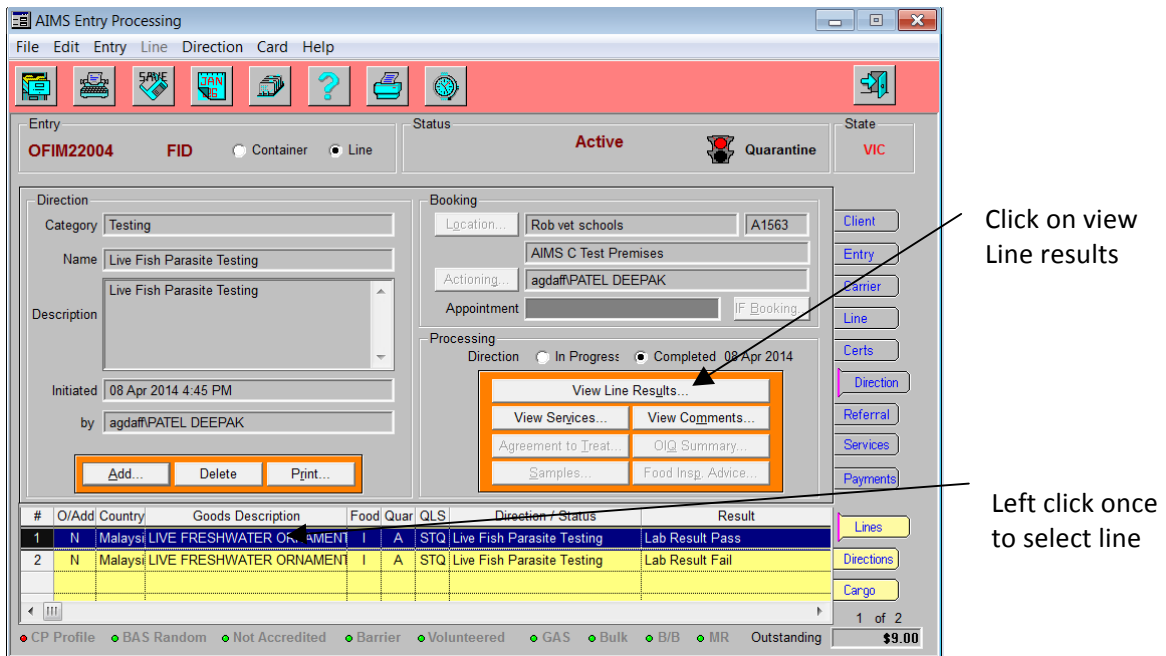


Fig 11: assigning lab results to line

11) From the available results choose the outcome of the test (e.g. Lab Result Pass) by left clicking once on the result you wish to assign and then left clicking once on the assign results button. The lab result will now appear in the middle (results) box next to the relevant line number. Click OK to finalise lab results.

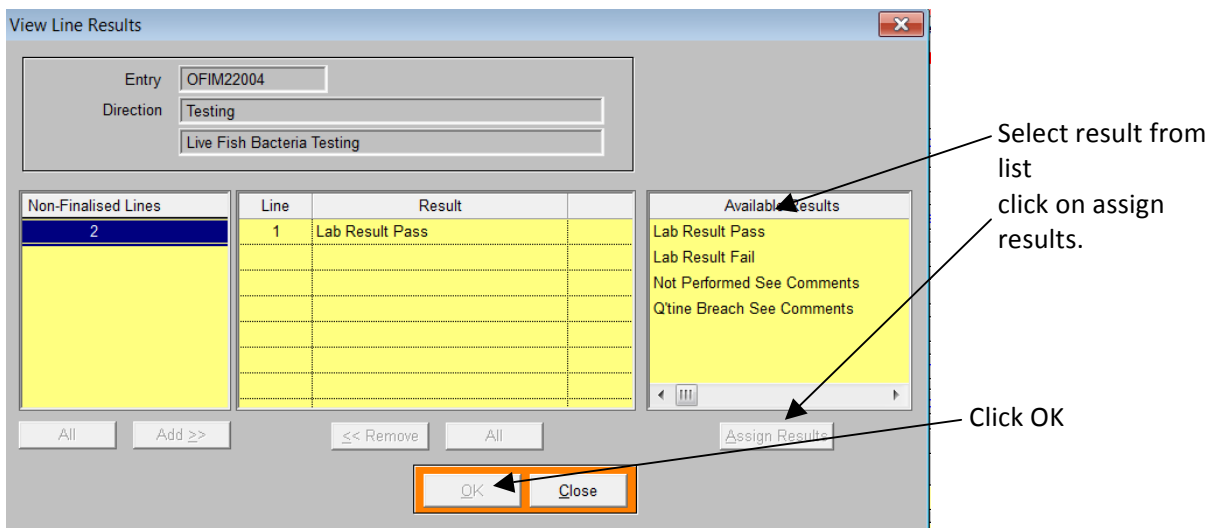
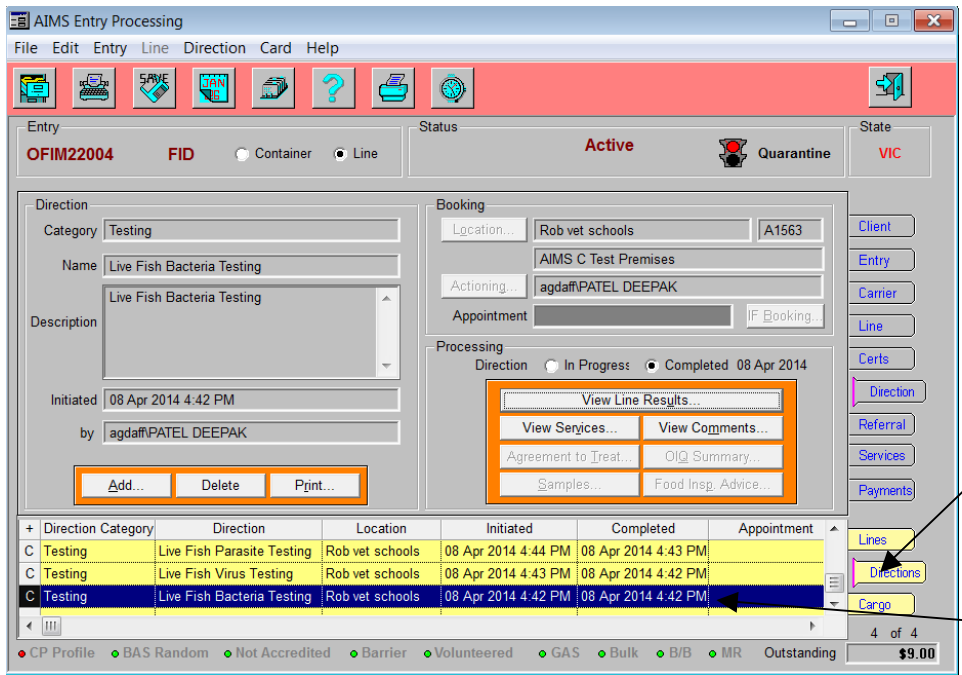


Fig 12: assign lab results to line

12) The test will now show as being completed in the line directions tab (bottom right hand side of screen).



Click Directions

Test completion time and date

Fig 13: viewing lists of tests performed

6.4 Identification of compliant fish bags for sampling

There are two types of bags to be sampled during this trial. One is the non-compliant/seized bag and another is the compliant/healthy bag.

Compliant/healthy bags are subject to random sampling and testing. Following the review of AIMS directions, the duty officer determines which risk group of fish is sampled for which specific testing.

New testing directions are as follows:

- Live Fish Virus Testing
- Live Fish Bacteria Testing
- Live Fish Parasite Testing

The results required on each testing direction are as follows:

- Lab Result Negative (Pass)
- Lab Result Positive (Fail)
- Not Performed See Comments - Pass
- Quarantine Breach See Comments - Fail

Table 3: Types of testing and directions for compliant/health bags

Risk group	Risk Group 1 Goldfish	Risk Group 2 Gourami [footnote subfamily etc]	Risk Group 3 Cichlid	Risk Group 4 Poeciliid	Risk Group 5 All other freshwater species
Type of testing and direction	SVCV Direction: Live Fish Virus Testing	Megalocytivirus Direction: Live Fish Virus Testing	Megalocytivirus Direction: Live Fish Virus Testing	Megalocytivirus Direction: Live Fish Virus Testing	Ectoparasite Direction: Live Fish Parasite Testing
	Aeromonas salmonicida Direction: Live Fish Bacteria Testing				
	Ectoparasite Direction: Live Fish Parasite Testing	Ectoparasite Direction: Live Fish Parasite Testing	Ectoparasite Direction: Live Fish Parasite Testing	Ectoparasite Direction: Live Fish Parasite Testing	

6.5 Selecting of the box for sampling

Use the ‘random number generator’ software on the dashboard to select which box number of fish among all boxes of the targeted risk group should be sampled randomly.



Enter the total number of boxes (from the packing list) into the random number generator and press ‘Select Random Box’. For example, if the consignment consists of 40 boxes containing risk group 1 (goldfish), risk group 2 (gouramis) and risk group 3 (cichlids), you key in the ‘Number of Boxes’ space. It will generate random number, 16 in the example above. For each fish group that is required to be sampled, the officer then needs to select a box number that is closest to the number 16 that has this fish group.

6.6 Selection of individual fish for sampling

During this trial, the importer’s staff will take the fish samples, place them into the sample container containing the Aqu-S solution pre-prepared by the duty officer. The the importer’s staff will re-aerate and re-

seal the bag of remaining fish under the supervision of the duty officers. The importer will provide all necessary equipment (other than those listed in Section 6.8.2) to re-aerate and re-seal the fish bag.

6.6.1 Compliant/healthy bags of poeciliids, gouramis and cichlids

Select five fish per risk group for sampling to test for *Megalocytivirus* and ectoparasites. The five fish are first examined for the presence of ectoparasites as per Section 6.9 by OSP and then sent to AAHL for virus testing.

Do not select fish that have died prior to inspection. Fish that are seen to die during inspection can be selected.

6.6.2 Compliant/healthy bags of goldfish

Select five fish for SVCV and ectoparasitology and another five fish for *A. salmonicida* testing (total ten fish). The five fish for testing *A. salmonicida* must be sent to AAHL on the same day. The other five fish are first examined for the presence of ectoparasites as per Section 6.9 by OSP and then sent to AAHL for virus testing. Do not select fish that have died prior to inspection. Fish that look sick and/or are about to die can be selected.

6.6.1 Compliant/healthy bags of other freshwater fish species

Select five fish for sampling. The five fish are examined for the presence of ectoparasites as per Section 6.9 by OSP.

Do not select fish that have died prior to inspection. Fish that look sick and/or are about to die can be selected.

6.7 Specimen Preparation

6.7.1 Sample identification and numbering

Five fish go to make up one sample. The sample should be given a separate number with regards to the sample preparation and labelling. For example, if a consignment has goldfish and gouramis being targeted for sampling, that consignment will have a total 2 samples and two corresponding sample submission numbers. For the goldfish, there will be the sample submission number plus 'a' and 'b' labels (one for virus testing and the other for bacteriology). For the gouramis, there will be one sample submission number.

Goldfish example: Goldfish: Spring Viraemia of Carp Virus sample – 203861a
Goldfish: *Aeromonas salmonicida* sample – 203861b

These numbers should be written on the sample containers (where applicable – if fresh fish, write on the outside of the bag). For samples sent to AAHL, the sample submission number should be written on the *AFDL Specimen Advice Note* (AAHL Submission Form – Appendix 1) in the “*Submitter Reference Number (optional)*” section for reconciliation of testing results from AAHL with the samples taken. Similarly, samples sent to the Victorian DEPI laboratory, the sample submission number should be written on the *DEPI Victoria Request for Laboratory Examination* form in the “*Animal Identification*” section.

Ensure the specific test and sample type (e.g. whole fish for *Aeromonas salmonicida* testing) is written on both of the laboratory specimen submission form in the “*Examination Requested*” section in the AAHL’s form and in the “*Investigations requested*” in the Victorian form.

Ensure the “*Submitter*” details are filled in appropriately and that the “*Reason for Submission*” (AAHL) and “*Nature and No. of Specimens*” (Victoria) sections list “*OFIR Trial 2*” as in Ornamental Fish Import Reform Trial 2. Examples are shown in Appendices 1 and 2.

6.7.2 Preparation of fish for specific tests

OSP will process the fish for testing; however, OSP may also transfer these activities to officers with appropriate skills.

Details of sampling methods for the specific pathogens and the risk groups of fish are below and in Table 4.

The materials and equipment used for processing and testing include:

- 80 % ethanol fixing solution
- 10 % formalin fixing solution
- AQUI-S solution for euthanasia
- Sampling equipment including disposable plastic cups (or sterilised nets), disposable gloves, new sample bags, new rubber bands to seal bags, new sample containers
- Preparation equipment including disposable scalpels, tweezers, pins for pinning fish to tray during dissection, Styrofoam trays and aluminium foil
- Ethanol for cleaning
- Paper towels
- Parafilm for sealing transport tubes/jars
- Zip lock or heat sealed bags
- Ice
- Eskies
- Large containers to transport sample bottles in
- Containers to hold fish

Note: Officers with appropriate skills can process the fish for testing under OSP guidance.



6.8 Euthanasia of fish

The officers should inform OSP at the end of the on-arrival inspection that there are fish to be sampled. The officers should not euthanise the fish until they have consulted with OSP and it has been agreed that OSP can immediately process the fish. If fish are ready for sampling after 3.30pm, inform OSP of their presence.

If there are non-compliant goldfish on a Friday, animals cannot be sampled. In this case, non-complaint goldfish should be destroyed as per standard instructions. For animal welfare reasons, do not hold these bags over the weekend.

Ask the importer's staff to use a net to capture the fish for sampling and placing in a container of water. The net is disinfected prior to use for another bag.

Euthanise the sampled fish as per Section 6.8.2 or the reference material *Euthanasia of ornamental fish*. Using Appendix 3, identify which fish risk group the bag of sampled fish belong to and using Table 4, confirm the sample preparation method required for the specific tests to which the fish samples will be subject.

6.8.1 Reagents and solutions

AQUI-S®

AQUI-S® is an aquatic anaesthetic that has been developed to give flexibility and control in animal husbandry, transporting and harvesting operations. The ingredients are approved for use in foods and AQUI-S® is safe for use in food fish. For euthanasia of fish 150 mg/l is recommended.

Make up a **stock solution** by diluting the concentrated AQUI-S® solution 1:10 with water (tap water is fine). Stock solution is an oily white emulsion that will only keep for 6 hours. The stock solution is shaken well to create the emulsion that is then added to the sample bags.

For example, if there are 10 sample bags to treat, each containing about 1L of water - that would be a total of 10L.

The final concentration required is about 175 mL/m³

The final volume is 10L, which is 0.01 m³

Therefore, the volume of AQUI-S concentrate required is 175 x 0.01 = 1.75 mL

Take a syringe and add 1.8 mL AQUI-S® to 18 mL of tapwater, shake well.

Use this stock solution at the rate of 1.8 mL per 1L bag of fish.

80% Ethanol

Ethanol is used as a preservative for tissues destined for molecular testing, where the quality of the DNA must be conserved.



10% Formalin

Formalin is a fixative used for the long term storage of tissues and for tissues intended for processing for histology. **Use gloves and use in a ventilated area to avoid inhaling concentrated fumes. Use safety glasses and/or a full face shield where splashing is possible.**

6.8.2 Euthanising fish with AQUI-S®

1. AQUI-S® can be used to euthanise the fish using a concentration of 150 -175 mL/m³. The aim is to use sufficient anaesthetic to sedate the fish within 5 -10 minutes.
2. Use surgical gloves.
3. Prepare the stock solution as per Section 6.8.1.
4. Open the sample bag and add in the required amount of AQUI-S®. Accuracy of measurement is not necessary.
5. Close the bag as the fish will become agitated.
6. Once the fish have become moribund, they can be sampled as follows.
7. If concern that AQUI-S® will dislodge parasites, then the water containing the fish when anaesthetised can be filtered through the plankton net (~ 75µm) into 70mL container and fixed with 2% formalin.

6.8.3 Preserving whole fish in 80 % ethanol

1. Prepare and label a small plastic container of sufficient size to hold the sampled fish together with at least 10 times the volume of ethanol as there is fish.
2. Use surgical gloves.

3. Open the sample bag and net out five euthanised fish. For larger fish if it is possible to safely do so, first slit the belly to allow the alcohol to enter the fish. For small fish, make a partial cut or stab through the belly.
4. Place in the plastic container of ethanol and put aside for ectoparasite examination.



6.8.4 Preserving whole fish in 10 % formalin

1. **Use surgical gloves and a well ventilated area.** Prepare and label a small plastic container of sufficient size to hold the sampled fish together with at least 10 times the volume of formalin as there is fish.
2. Open the sample bag and net out five euthanised fish. For larger fish if it is possible to safely do so, first slit the belly to allow the formalin to enter the fish. For small fish, make a partial cut or stab through the belly.
3. Place in the plastic container of formalin and put aside for ectoparasite examination.

6.9 Ectoparasitology by OSP

6.9.1 Laboratory processing fixed samples for ectoparasites (in 10 % formalin or 80 % ethanol)

1. Shake sample container for 30 seconds to 1 minute.
Intent: to dislodge any ectoparasite that may be attached to the fish.
2. Remove fish and examine host body surface by eye, or under dissecting scope, for ectoparasites. Focus on body surface and fins as these areas are the main habitats for ectoparasites.
Intent: to observe and identify ectoparasites that remain attached to host.
3. Pour wash from sample container into a petri dish and examine under dissecting scope.
Intent: it is easier to observe and identify ectoparasites in petri dish than when attached to the fish. Small, less mature ectoparasites are likely to have detached from the fish and can be found in this wash. If volume of this wash is large (for instance greater than several hundred millilitres), it can be filtered through a 75 µm filter — note that wash is very quickly processed when it is clean and contains little debris or few ectoparasites.
4. Dissect out second gill arch from a gill and examine under a dissecting scope. Gill tissue is best observed when submerged in water.
Intent: to observe and identify gill flukes that have remained attached to gill tissue. These results could be compared against histology from the corresponding gill arch of the second gill.
5. For healthy fish, observe and identify those ectoparasites determined by the Ornamental Finfish IRA 1999 to require management.
Intent: ectoparasites of concern include *Dactylogyrus vastor* and *D. Extensus*, *Argulus foliaceus* and *A. coregoni* and *Lernaea elegans*. The ectoparasites *Acanthamoeba* spp. and *Ergasilus sieboldi* may need to be included. Other parasite species may be found that are not considered of biosecurity significance.

6.9.2 Laboratory processing fresh samples for ectoparasites

At the beginning of the trial, OSP provide relevant officers with training to handle fresh samples for ectoparasite examination as follows:

1. Remove fish from anaesthetic.
2. Remove fish and examine host body surface [by eye or under dissecting scope] for ectoparasites. Focus on body surface and fins as these areas are the main habitats for ectoparasites.
Intent: to observe and identify ectoparasites that remain attached to host. Ectoparasites are easier to observe when tissue is fresh because they move.
3. Dissect out second gill arch from a gill and examine under a dissecting scope. Gill tissue is best observed when submerged in water.
Intent: observe and identify gill flukes that have remained attached to gill tissue. These results could be compared against histology from the corresponding gill arch of the second gill. Ectoparasites are easier to observe when tissue is fresh because they move.
4. Fix fish in ethanol or formalin and send to laboratories for further testing.

6.9.3 Recording of results

1. Count number of individuals within a group of like (family/genus/species) specimens.
 - a. It is sufficient when examining host and filtrate to group like pathogens and count individuals within the group. The group of like specimens can be identified to species later.
2. Please denote on data sheet if number is actual or estimated.
 - a. For instance, if number of pathogens are too numerous to count with accuracy, then estimate to a log value, like 10's, 100's or 1000's. If number is estimated, please also denote estimate method (log, range, category).
3. Identify group of like pathogens to:
 - a. Family/Genus;
 - b. Species if genus is *Dactylogyrus*, *Argulus*, *Lernaea*, *Acanthamoeba* (the identification of *Acanthamoeba* is optional) or *Ergasilus*.

	Species	Total (#/est)	Juvenile (#/%)	Adult (#/%)
Ectoparasites of Concern	<i>Dactylogyrus vastor</i>			
	<i>Dactylogyrus extensus</i>			
	<i>Argulus foliaceus</i>			
	<i>Argulus coregoni</i>			
	<i>Lernaea elegans</i>			
	<i>Acanthamoeba</i> spp.			
	<i>Ergasilus sieboldi</i>			
Ectoparasites	Has been assessed for			

not of Concern	disease risk (in IRA)			
	Hasn't been assessed for disease risk			

6.9.4 Reporting results and interpretation

For consignment of fish deemed health at border by a biosecurity officer

1. Ectoparasites of concern
 - a. Are present along pathway and require active management to meet import conditions. Their total number will reflect how well they are being managed. A well managed system will be free of these ectoparasites. Freedom of these ectoparasites can be achieved by breaking their direct life cycle through implementation of strategic treatments. These treatments should be documented and will form part of their auditable documentation.
2. Ectoparasites not of concern
 - a. Are indicators of application and efficiency of pre-export treatment. Depending on wording of our import requirements, do we require pre-export treatment or fish free of the particular ectoparasite?

For consignment of fish deemed unhealthy at border by a biosecurity officer

- a. Is this pathogen of biosecurity concern? Cross-reference pathogen against ectoparasites that have already been assessed in IRA. If has already been assessed, then take no further action. If pathogen has not previously been assessed, assess biosecurity risks for possible inclusion onto list of ectoparasites of concern.

6.10 Preparing fish for transportation to AAHL and the Victoria DEPI laboratory

Following ectoparasitology by OSP, the fish will be further processed for transportation to AAHL for bacteria and virus PCR testing and the Victoria DEPI laboratory for histopathological examination.

When processing the fish be sure to keep the area as clean as possible. Do not use the same items between fish without flaming in alcohol first. Also be aware of not using dissection tools on the “outside” and “inside” of the fish without flaming with alcohol between use. Be sure to cool the tools after flaming and before using again.

Table 4: Brief guide to preparing sampled fish for transportation to laboratories

Fish group	Total fish required to be sampled per bag	Specific test	Sample type	Brief guide to processing samples (see individual sections for full instructions regarding processing of fish for each specific test)
Compliant/healthy bag of Goldfish	5	<i>Aeromonas salmonicida</i>	Whole fresh fish (If fish are >6cm) Dissected organs (including gill)	Pat dry, bag, put on wet ice. Transport to AAHL immediately.
	5	Ectoparasitology and SVCV	Whole fish (If fish are >6cm) Dissected organs (including gill)	Place in tube containing 80% ethanol. The volume of 80% ethanol must be at least 10 times the volume of the specimen
Compliant/healthy bag of Poeciliids, Gouramis and Cichlids	5	Ectoparasitology and Iridovirus (<i>Megalocytivirus</i>)	Whole fish (If fish are >6cm) Dissected organs (including gill)	Place in tube containing 80% ethanol. The volume of 80% ethanol must be at least 10 times the volume of the specimen
Compliant/healthy bag of Other freshwater fish	5	Ectoparasitology	Whole fish (If fish are >6cm) Dissected organs (including gill)	Place in tube containing 80% ethanol. The volume of 80% ethanol must be at least 10 times the volume of the specimen

Fish group	Total fish required to be sampled per bag	Specific test	Sample type	Brief guide to processing samples (see individual sections for full instructions regarding processing of fish for each specific test)
Non-compliant/seized bag of Goldfish	5	<i>Aeromonas salmonicida</i>	Whole fresh fish (If fish are >6cm) Dissected organs (including gill)	Pat dry, bag, put on wet ice. Transport to AAHL immediately.
	5	Histology	Tail off and slit open along body cavity	Place in tube containing 10% formalin. The volume of 10% formalin must be at least 10 times the volume of the specimen
	5	SVCV	Whole fish (If fish are >6cm) Dissected organs (including gill)	Place in tube containing 80% ethanol. The volume of 80% ethanol must be at least 10 times the volume of the specimen
Non-compliant/seized bag of Poeciliids, Gouramis and Cichlids	5	Histology	Tail off and slit open along body cavity	Place in tube containing 10% formalin. The volume of 10% formalin must be at least 10 times the volume of the specimen
	5	Iridovirus (<i>Megalocytivirus</i>)	Whole fish (If fish are >6cm) Dissected organs (including gill)	Place in tube containing 80% ethanol. The volume of 80% ethanol must be at least 10 times the volume of the specimen
Non-compliant/seized bag of All other species including marines	5	Histology	Tail off and slit open along body cavity	Place in tube containing 10% formalin. The volume of 10% formalin must be at least 10 times the volume of the specimen

6.10.1 Sample preparation for *A. salmonicida* testing

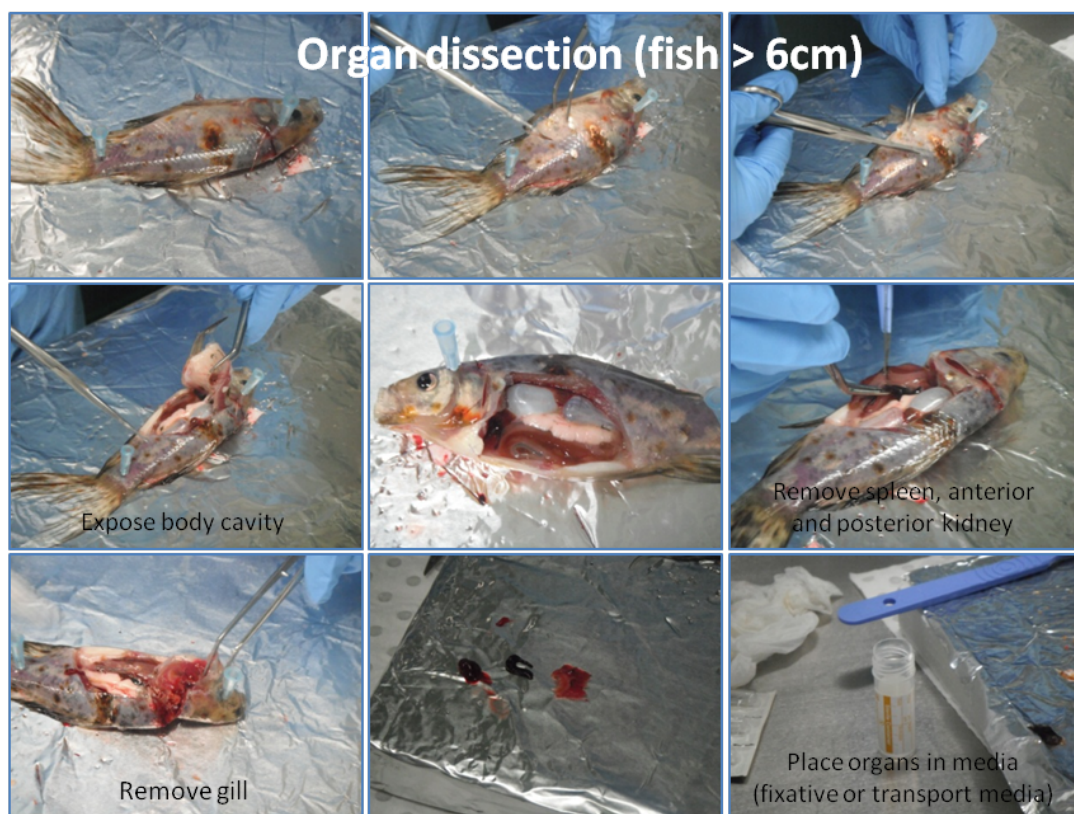
Once the fish has been euthanised, pat it dry with clean paper towel, place it in a sample bag and then place in wet ice. The goldfish samples for *Aeromonas salmonicida* testing must be couriered to AAHL at 4°C on wet ice on the day the fish is sampled. Fresh tissue/fish must be processed by the laboratory within 24 hrs of the fish being euthanised. This time constraint must be considered against courier times to allow the laboratory to carry out their work against the time limitation - the officer responsible for transportation of fish samples should directly liaise with AAHL about arrangements.

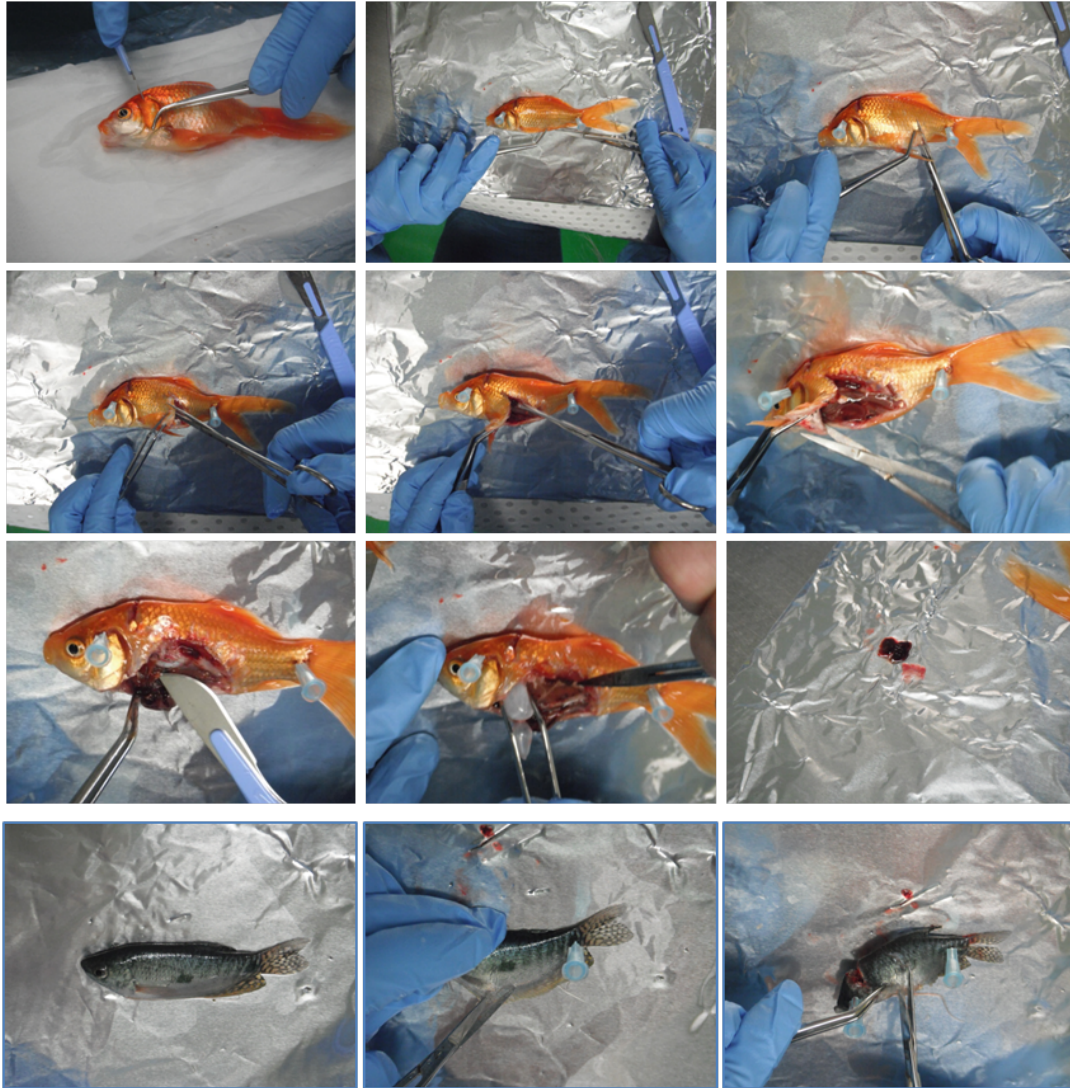
6.10.2 Sample preparation for Spring Viraemia of Carp Virus (SVCV) testing

Following ectoparasitology, the fish are prepared for transportation to AHHL for SVCV testing. For fish less than 6 cm, whole, tail-off euthanised fish can be submitted to the testing laboratory in 80% ethanol. Fish should be euthanised, the tail taken off and the body cavity cut open to allow penetration of the fixative. The fish should be put in a small tube with the 80% ethanol and labelled with the appropriate sample number and individual identifying number for the fish. The volume of fixative must be at least 10 times the volume of the specimen.

For fish longer than 6 cm, a pool of kidney (anterior and posterior), spleen and gill tissue should be taken from the fish. Use the pins to keep the fish attached to the Styrofoam sampling board. Slice the fish open along the body cavity and remove the flap across the body cavity to expose the organs. Once body cavity organs are exposed, use a new scalpel to remove the organs (see pictures below – *Organ dissection (fish >6cm)*). This pool of organs should be put in a tube containing 80% ethanol and labelled with the appropriate sample number and individual identifying number for the fish. The volume of fixative must be at least 10 times the volume of the specimen.

The fixed fish can be stored by OSP in appropriate space until storage space for a number of fish samples becomes an issue. Approximately 20 fixed samples or more as a transportation batch can be sent to AAHL by overnight couriers on a regular basis – the officer responsible for transportation of fish samples should directly liaise with AAHL about arrangements.





6.10.3 Sample preparation for Megalocytivirus testing

Following ectoparasitology, the fish are prepared for transportation to AHHL for Megalocytivirus testing. For fish less than 6 cm, whole, tail-off euthanised fish can be submitted to the testing laboratory in 80% ethanol. Fish should be euthanised, the tail taken off and the body cavity cut open to allow penetration of the fixative. The fish should be put in a small tube with the 80% ethanol and labelled with the appropriate sample number and individual identifying number for the fish. The volume of fixative must be at least 10 times the volume of the specimen.

For fish longer than 6 cm, a pool of kidney (anterior and posterior), spleen and gill should be taken from the fish. Use the pins to keep the fish attached to the Styrofoam sampling board. Slice the fish open along the body cavity and remove the flap across the body cavity to expose the organs. Once body cavity organs are exposed, use a new scalpel to remove the organs (see pictures above – *Organ dissection (fish >6cm)*). This pool of organs should be put in a tube containing 80% ethanol and labelled with the appropriate sample number and individual identifying number for the fish. The volume of fixative must be at least 10 times the volume of the specimen.

The fixed fish can be stored by OSP in appropriate space until storage space for a number of fish samples becomes an issue. Approximately 20 fixed samples or more as a transportation batch can be sent to AAHL by overnight couriers on a regular basis – the officer responsible for transportation of fish samples should directly liaise with AAHL about arrangements.

6.10.4 Sample preparation for histology

For fish less than 6 cm, whole, tail-off euthanised fish can be submitted to the testing laboratory in 10% formalin. Fish should be euthanised, the tail taken off and the body cavity cut open to allow penetration of the fixative (see photo below – *Sample preparation for histology (fish <6cm)*). The fish should be put in a small tube with the 10% formalin and labelled with the appropriate sample number and individual identifying number for the fish. The volume of fixative must be at least 10 times the volume of the specimen.

For fish > 6 cm, a pool of kidney (anterior and posterior), spleen and gill should be taken from the fish. Use the pins to keep the fish attached to the Styrofoam sampling board. Slice the fish open along the body cavity and remove the flap across the body cavity to expose the organs. Once body cavity organs are exposed, use a new scalpel to remove the organs (see pictures above – *Organ dissection (fish >6cm)*). This pool of organs should be put in a tube containing 10% formalin and labelled with the appropriate sample number and individual identifying number for the fish. The volume of fixative must be at least 10 times the volume of the specimen.

The fixed fish can be stored in appropriate space until storage space for a number of fish samples becomes an issue. Approximately 20 fixed samples or more as a transportation batch can be sent to the Victoria DEPI laboratory by overnight carriers on a regular basis – the officer responsible for transportation of fish samples should directly liaise with the laboratory about arrangements.



6.11 Disposal of waste material

Dispose of all quarantine risk material and disposable equipment in the quarantine waste bins located in the in-house inspection room and/or in appropriate sharps-containers. Fish tissue waste should be stored in a freezer in a bag clearly labelled as “Quarantine waste”. The bag should be disposed of in the quarantine waste bins immediately prior to their emptying. This will prevent any odours occurring. In addition, clean all surfaces and reusable equipment used in sampling and preparation with either ethanol or other effective disinfectant at the appropriate rate.

6.12 Recommended carriers

AAHL recommend using *Geelong Document & Parcel Express Pty Ltd*. The courier will need to send their invoices directly to AAHL for payment, either electronically or via post:

Post: CSIRO AAHL, Private Bag 24, Geelong, VIC 3220

The Victoria DEPI laboratory prefers Toll as their courier. The laboratory has an account with Toll, the account code 3004GR (AgriBio Bundoora – DEPI)

Post: DPI VICTORIA – Veterinary Diagnostic Services, AgriBio Specimen Reception, 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083

Phone: 03 9032 7515

Fax: 03 9032 7604

It is the SER officer’s responsibility to liaise with the couriers to ensure that laboratories are notified of incoming specimens and that the sample will be delivered to meet their arrival time requirements. The officer who sends samples needs to ask laboratories to send the result back to “SERO Generic fish team email box”.

Paperwork required to accompany the consignment includes a copy of the Quarantine Entry, the *AFDL Specimen Advice Note* (Appendix 1) for the samples sent to AAHL and the *DEPI Victoria Request for Laboratory Examination* form (Appendix 2) for the samples sent to the Victoria DEPI laboratory.

Place paperwork in an envelope taped to the top or side of the consignment or contained in a zip lock bag inside the esky.

Fill in OSP's *Movement of Quarantine Material* spreadsheet (located in the *Health monitoring Trial* folder in the *Scientific (OSP-PEPQ)* folder on SER J: drive).

7 Laboratory results and recording in AIMS

The laboratory test report must include on a single document: a definitive statement regarding whether testing has resulted in a positive or negative result. The laboratory should be contacted if the test results are not in this format.

The officer or a person authorised on their behalf, accesses the AIMS database and locates the AIMS entry that is subject of the particular test result to be recorded. While in the AIMS database, enter test result details.

To enter in lab test results into the AIMS database the officer or person authorised should follow the following instructions:

1) click on the Directions tab at the bottom of the screen and highlight a line of interest. Then select the Direction tab on the middle right hand side of the screen to bring up the screen which shows further details in relation to the direction selected.

2) click view line results to bring up the screen in which you can assign lab results.

Select 'Direction' tab

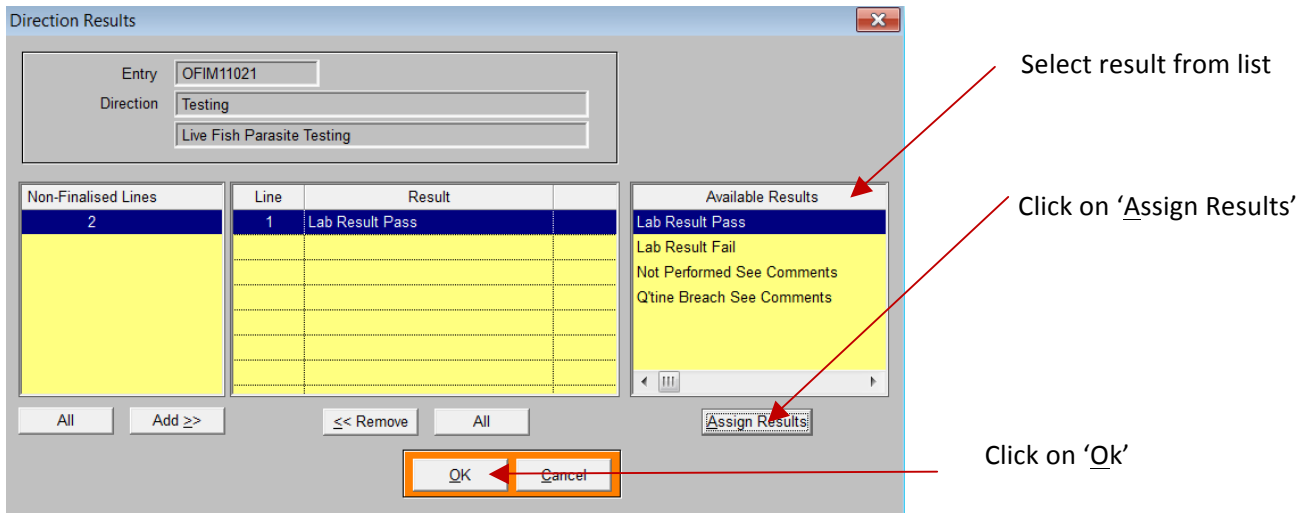
Select 'Directions' tab To view directions

Highlight line of interest

Direction Category	Direction	Location	Initiated	Completed	Appointment
C Testing	Live Fish Parasite Testing	Rob vet schools	08 Apr 2014 4:45 PM	08 Apr 2014 4:44 PM	
C Testing	Live Fish Parasite Testing	Rob vet schools	08 Apr 2014 4:44 PM	08 Apr 2014 4:43 PM	
C Testing	Live Fish Virus Testing	Rob vet schools	08 Apr 2014 4:43 PM	08 Apr 2014 4:42 PM	

3) After having highlighted the line of interest by clicking on it once, proceed to click on 'view line results' to bring up the screen where you will be able to assign lab results. The results required on each testing direction are as follows:

- Lab Result Negative (Pass)
- Lab Result Positive (Fail)
- Lab Histo Result Q'tine Concern (Fail)
- Lab Histo Result No Q'tine Concern (Pass)
- Not Performed See Comments
- Quarantine Breach See Comments



When the finalised and released direction is added, the officer needs to explicitly state in the comment area that the remains of line X, Y, Z not sent for testing, are also released from Quarantine. This comment will be printed out on the finalised and released direction that the client receives.

The original test result should be kept with the consignment paperwork and a copy emailed to the Department.

8 Work health and safety

This I&G requires the department's biosecurity officers to examine live fish. Some fish species can cause injury due to sharp spines and care must be taken. Live fish are inspected at Air Cargo Terminals or at the Department's regional office facilities and biosecurity officers must adhere to all WH&S procedures at these facilities.

It is a requirement that biosecurity officers performing examinations under this I&G have had induction training that includes WH&S awareness. Water proof gloves must be worn and protective apron or coat or sleeve protectors should be worn during inspection of live fish.

Refer to for details of the department's WH&S policy and incident reporting tools.

9 Roles and responsibilities

Authorised quarantine officers are responsible for:

- examining documentation to determine if the level of risk is acceptable or unacceptable
- examining the fish to determine if the level of risk is acceptable or unacceptable
- seeking technical advice from the Animal Import Operations Branch as required
- ordering animals into quarantine
- issuing quarantine directions
- initiate a pest interception form
- notifying importers or agents of decisions

- draw sample for testing
- euthanize fish (OSP)
- complete a PDI form
- initiate incident in Incidents Client database
- update AIMS entry with relevant details and directions
- complete specimen advice (OSP)
- update and complete incident in Incidents Client database (OSP)
- prepare sample (OSP)
- submit sample (OSP)
- record test results in Incidents Client and forward copy to the Department and CEBRA (OSP)
- support officers involved in Trial One (OSP)

Animal Import Operations Branch is responsible for:

- understanding the animal health risks associated with fish
- assessing and issuing import permits
- providing technical animal health advice to other Departmental officers as required
- issuing industry notices as required
- maintaining ICON
- updating regional Departmental biosecurity officers on new information as required.

Animal Biosecurity Branch is responsible for:

- providing health monitoring testing advice to Departmental Biosecurity officers as required
- collating and analysing laboratory results
- listing laboratories approved for testing

10 Record keeping

Copies of all documents associated with, and generated during, the on-arrival inspection process must be kept at the regional office as per Departmental record keeping requirements. All electronic documents including all emails relating to this trial should be saved in the *Health monitoring Trial* folder in the *Scientific (OSP-PEPQ)* folder on SER J: drive.

11 Related material

It is the responsibility of the officer to ensure they are accessing the most up to date information. This I&G refers to the following materials which are available from the Department of Agriculture Instructional Material Library:

- Standard Operating Procedure - *Quarantine management of imported goods*;
- Reference material - *Euthanasia of ornamental fish*;
- Guideline material: *Fish taxonomy- a guide to understanding the permitted species list*;
- Instruction and Guideline - *Post-entry quarantine of live ornamental fish at class 7.1 Quarantine Approved Premises*;
- Instruction and Guideline - *Actions and outcomes for non-compliances arising from on-arrival inspection of documents and ornamental fish consignments*; and
- Reference – *Creating and Incident*.

The following associated materials may be found on the department's website and ICON:

- [ICON](#) - *Import condition for live ornamental freshwater or marine fish*

12 Detailed version history

Version Number	Version Date	Amendment Details
One	21/02/2014	Initial draft
Two	28/02/14	Include comments on initial draft
Three	31/03/14	Include ectoparasitology procedures
Four	2/05/14	Include Dashboard and AIMS instructions
Five	7/05/14	FINAL

13 Appendices

Appendix 1 *AFDL Specimen Advice Note* (AAHL laboratory submission form)

Appendix 2 *DEPI Victoria Request for Laboratory Examination Form*

Appendix 3 List of approved species and risk group

APPENDIX 1

AFDL SPECIMEN ADVICE NOTE

CSIRO Animal, Food and Health Sciences (CAFHS)
 Australian Animal Health Laboratory (AAHL)
 AAHL Fish Diseases Laboratory
 Private Bag 24, Geelong, Vic 3220
 Telephone: + 61 3 5227 5000
 Fax: + 61 3 5227 5555
 Email: afdl@csiro.au



AAHL SAN Number (AAHL Use Only)	Page 1 of 1 QA/14.2.9
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<p><u>Submitter Reference Number (optional):</u></p> <div style="border: 1px solid black; height: 20px; width: 100%;"></div> <p><u>Submitter:</u></p> <p>State/Country:</p> <p>Email: <div style="border: 1px solid black; width: 80%;"></div></p> <p>Telephone (business):</p> <p>Telephone (after hours):</p> <p>FAX:</p> <p><u>Forward account to:</u></p> <p><u>Send additional copies of reports to:</u></p>	<p><u>Submission Category (circle one):</u></p> <p>1 = Routine Submission 2 = Emergency Disease Exclusion (low probability) 3 = Emergency Disease Exclusion (high probability)</p> <p><u>Animal details:</u></p> <p>Species:</p> <p>Date specimens collected:</p> <p>Age/Year class: Farmed <input type="checkbox"/> Wild <input type="checkbox"/></p> <p>Sex:</p> <p>Farm or location:</p> <p>Owner:</p> <p>Origin of population:</p> <p>Population size:</p> <p>Marine zone/Hatchery:</p> <p><u>Water conditions:</u></p> <p>Temperature:</p> <p>Salinity:</p> <p>Other:</p>
<p><u>Reason for submission:</u></p>	
<p><u>Specimen details:</u></p>	
<p><u>History and clinical signs:</u></p>	
<p><u>Examination requested:</u></p>	<p><u>AFDL Use only</u></p>
<p><u>Signature of submitter (please also print name):</u></p>	<p>Date of dispatch to AAHL:</p> <p>Date specimens received at AAHL:</p>

Author	Bernadette O'Keefe	14 th August 2012
Authorised by	Mark Crane	
Published by	Bernadette O'Keefe	



DPI VICTORIA – Veterinary Diagnostic Services
AgriBio Specimen Reception
 5 Ring Road, La Trobe University.
 Bundoora, Victoria, 3083
 Phone: (03) 9032 7515 Fax: (03) 9032 7604
 Email: vet.diagnostics@dpi.vic.gov.au



Number: 14477

Tube No.	Name or ID no.	Brand	Tube No.	Name or ID No.	Brand
1			51		
2			52		
3			53		
4			54		
5			55		
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8			58		
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10			60		
11			61		
12			62		
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50			100		

Appendix 3

List of approved species and risk group

Family	sub-Family	Genus (accepted)	Permitted Taxon	Risk Group
Acanthuridae		<i>Acanthurus</i>	<i>Acanthurus</i> spp.	Marine
Acanthuridae		<i>Ctenochaetus</i>	<i>Ctenochaetus</i> spp.	Marine
Acanthuridae		<i>Naso</i>	<i>Naso</i> spp.	Marine
Acanthuridae		<i>Paracanthurus</i>	<i>Paracanthurus</i> spp.	Marine
Acanthuridae		<i>Prionurus</i>	<i>Prionurus</i> spp.	Marine
Acanthuridae		<i>Zebrasoma</i>	<i>Zebrasoma</i> spp.	Marine
Achiridae		<i>Trinectes</i>	<i>Trinectes maculatus</i>	Other Freshwater
Adrianichthyidae	Oryziinae	<i>Oryzias</i>	<i>Oryzias latipes</i>	Other Freshwater
Alestidae		<i>Alestopetersius</i>	<i>Alestopetersius caudalis</i>	Other Freshwater
Alestidae		<i>Arnoldichthys</i>	<i>Arnoldichthys spilopterus</i>	Other Freshwater
Alestidae		<i>Brycinus</i>	<i>Brycinus longipinnis</i>	Other Freshwater
Alestidae		<i>Lepidarchus</i>	<i>Lepidarchus adonis</i>	Other Freshwater
Alestidae		<i>Phenacogrammus</i>	<i>Phenacogrammus interruptus</i>	Other Freshwater
Ambassidae		<i>Chanda</i>	<i>Chanda</i> spp.	Other Freshwater
Anomalopidae		<i>Anomalops</i>	<i>Anomalops</i> spp.	Marine
Anomalopidae		<i>Kryptophanaron</i>	<i>Kryptophanaron</i> spp.	Marine
Anomalopidae		<i>Parmops</i>	<i>Parmops</i> spp.	Marine
Anomalopidae		<i>Photoblepharon</i>	<i>Photoblepharon</i> spp.	Marine
Anomalopidae		<i>Phthanophaneron</i>	<i>Phthanophaneron</i> spp.	Marine
Anomalopidae		<i>Protoblepharon</i>	<i>Protoblepharon</i> spp.	Marine
Anostomidae		<i>Abramites</i>	<i>Abramites hypselonotus</i>	Other Freshwater
Anostomidae		<i>Anostomus</i>	<i>Anostomus</i> spp.	Other Freshwater
Anostomidae		<i>Leporinus</i>	<i>Leporinus arcus</i>	Other Freshwater
Anostomidae		<i>Leporinus</i>	<i>Leporinus fasciatus</i>	Other Freshwater
Anostomidae		<i>Leporinus</i>	<i>Leporinus maculatus</i>	Other Freshwater
Anostomidae		<i>Leporinus</i>	<i>Leporinus multifasciatus</i>	Other Freshwater
Aplocheilidae		<i>Aplocheilus</i>	<i>Aplocheilus</i> spp.	Other Freshwater
Apogonidae		<i>Apogon</i>	<i>Apogon</i> spp.	Marine
Apogonidae		<i>Apogonichthyoides</i>	<i>Apogonichthyoides</i> spp.	Marine
Apogonidae		<i>Apogonichtys</i>	<i>Apogonichtys</i> spp.	Marine
Apogonidae		<i>Archamia</i>	<i>Archamia</i> spp.	Marine
Apogonidae		<i>Astrapogon</i>	<i>Astrapogon</i> spp.	Marine
Apogonidae		<i>Cercamia</i>	<i>Cercamia</i> spp.	Marine
Apogonidae		<i>Cheilodipterus</i>	<i>Cheilodipterus</i> spp.	Marine
Apogonidae		<i>Coranthus</i>	<i>Coranthus</i> spp.	Marine
Apogonidae		<i>Foa</i>	<i>Foa</i> spp.	Marine
Apogonidae		<i>Fowleria</i>	<i>Fowleria</i> spp.	Marine

Apogonidae		<i>Glossamia</i>	<i>Glossamia</i> spp.	Marine
Apogonidae		<i>Gymnapogon</i>	<i>Gymnapogon</i> spp.	Marine
Apogonidae		<i>Holapogon</i>	<i>Holapogon</i> spp.	Marine
Apogonidae		<i>Jaydia</i>	<i>Jaydia</i> spp.	Marine
Apogonidae		<i>Lachneratus</i>	<i>Lachneratus</i> spp.	Marine
Apogonidae		<i>Neamia</i>	<i>Neamia</i> spp.	Marine
Apogonidae		<i>Nectamia</i>	<i>Nectamia</i> spp.	Marine
Apogonidae		<i>Ostorhinchus</i>	<i>Ostorhinchus</i> spp.	Marine
Apogonidae		<i>Paxton</i>	<i>Paxton</i> spp.	Marine
Apogonidae		<i>Phaeoptyx</i>	<i>Phaeoptyx</i> spp.	Marine
Apogonidae		<i>Pseudamia</i>	<i>Pseudamia</i> spp.	Marine
Apogonidae		<i>Pseudamiops</i>	<i>Pseudamiops</i> spp.	Marine
Apogonidae		<i>Pterapogon</i>	<i>Pterapogon</i> spp.	Marine
Apogonidae		<i>Rhabdamia</i>	<i>Rhabdamia</i> spp.	Marine
Apogonidae		<i>Siphamia</i>	<i>Siphamia</i> spp.	Marine
Apogonidae		<i>Sphaeramia</i>	<i>Sphaeramia</i> spp.	Marine
Apogonidae		<i>Vincentia</i>	<i>Vincentia</i> spp.	Marine
Apogonidae		<i>Zoramia</i>	<i>Zoramia</i> spp.	Marine
Apterontidae		<i>Apteronotus</i>	<i>Apteronotus albifrons</i>	Other Freshwater
Apterontidae		<i>Apteronotus</i>	<i>Apteronotus leptorhynchus</i>	Other Freshwater
Auchenipteridae	Auchenipterinae	<i>Trachelyopterus</i>	<i>Trachelyopterus fisheri</i>	Other Freshwater
Bagridae		<i>Bagrichthys</i>	<i>Bagrichthys hypselopterus</i>	Other Freshwater
Bagridae		<i>Pseudomystus</i>	<i>Pseudomystus siamensis</i>	Other Freshwater
Balistidae		<i>Abalistes</i>	<i>Abalistes</i> spp.	Marine
Balistidae		<i>Balistapus</i>	<i>Balistapus</i> spp.	Marine
Balistidae		<i>Balistes</i>	<i>Balistes</i> spp.	Marine
Balistidae		<i>Balistoides</i>	<i>Balistoides</i> spp.	Marine
Balistidae		<i>Canthidermis</i>	<i>Canthidermis</i> spp.	Marine
Balistidae		<i>Melichthys</i>	<i>Melichthys</i> spp.	Marine
Balistidae		<i>Odonus</i>	<i>Odonus</i> spp.	Marine
Balistidae		<i>Pseudobalistes</i>	<i>Pseudobalistes</i> spp.	Marine
Balistidae		<i>Rhinecanthus</i>	<i>Rhinecanthus</i> spp.	Marine
Balistidae		<i>Sufflamen</i>	<i>Sufflamen</i> spp.	Marine
Balistidae		<i>Xanthichthys</i>	<i>Xanthichthys</i> spp.	Marine
Balistidae		<i>Xenobalistes</i>	<i>Xenobalistes</i> spp.	Marine
Balitoridae	Balitorinae	<i>Homaloptera</i>	<i>Homaloptera orthogoniata</i>	Other Freshwater
Balitoridae	Balitorinae	<i>Pseudogastromyz</i>	<i>Pseudogastromyzon myersi</i>	Other Freshwater
Bedotiidae		<i>Bedotia</i>	<i>Bedotia geayi</i>	Other Freshwater
Blenniidae	Blenniinae	<i>Meiacanthus</i>	<i>Meiacanthus atrodorsalis</i>	Marine
Blenniidae	Blenniinae	<i>Meiacanthus</i>	<i>Meiacanthus grammistes</i>	Marine
Blenniidae	Blenniinae	<i>Meiacanthus</i>	<i>Meiacanthus oualanensis</i>	Marine
Blenniidae	Salariinae	<i>Cirripectes</i>	<i>Cirripectes stigmaticus</i>	Marine
Blenniidae	Salariinae	<i>Ecsenius</i>	<i>Ecsenius axelrodi</i>	Marine
Blenniidae	Salariinae	<i>Ecsenius</i>	<i>Ecsenius bicolor</i>	Marine
Blenniidae	Salariinae	<i>Ecsenius</i>	<i>Ecsenius gravieri</i>	Marine
Blenniidae	Salariinae	<i>Ecsenius</i>	<i>Ecsenius melarchus</i>	Marine

Blenniidae	Salariinae	<i>Ecsenius</i>	<i>Ecsenius midas</i>	Marine
Blenniidae	Salariinae	<i>Lipophrys</i>	<i>Lipophrys nigriceps</i>	Marine
Blenniidae		<i>Ecseniuspulcher</i>	<i>Ecseniuspulcher</i>	Marine
Bythitidae		<i>Acarobythites</i>	<i>Acarobythites</i> spp.	Marine
Bythitidae		<i>Alionematichthys</i>	<i>Alionematichthys</i> spp.	Marine
Bythitidae		<i>Anacanthobythite</i>	<i>Anacanthobythites</i> spp.	Marine
Bythitidae		<i>Beaglichthys</i>	<i>Beaglichthys</i> spp.	Marine
Bythitidae		<i>Bellottia</i>	<i>Bellottia</i> spp.	Marine
Bythitidae		<i>Bidenichthys</i>	<i>Bidenichthys</i> spp.	Marine
Bythitidae		<i>Brosmodorsalis</i>	<i>Brosmodorsalis</i> spp.	Marine
Bythitidae		<i>Brosmolus</i>	<i>Brosmolus</i> spp.	Marine
Bythitidae		<i>Brosmophyciops</i>	<i>Brosmophyciops</i> spp.	Marine
Bythitidae		<i>Brosmophycis</i>	<i>Brosmophycis</i> spp.	Marine
Bythitidae		<i>Brotulinella</i>	<i>Brotulinella</i> spp.	Marine
Bythitidae		<i>Bythites</i>	<i>Bythites</i> spp.	Marine
Bythitidae		<i>Calamopteryx</i>	<i>Calamopteryx</i> spp.	Marine
Bythitidae		<i>Cataetyx</i>	<i>Cataetyx</i> spp.	Marine
Bythitidae		<i>Dactylosurculus</i>	<i>Dactylosurculus</i> spp.	Marine
Bythitidae		<i>Dermatopsis</i>	<i>Dermatopsis</i> spp.	Marine
Bythitidae		<i>Dermatopsoides</i>	<i>Dermatopsoides</i> spp.	Marine
Bythitidae		<i>Diancistrus</i>	<i>Diancistrus</i> spp.	Marine
Bythitidae		<i>Didymothallus</i>	<i>Didymothallus</i> spp.	Marine
Bythitidae		<i>Dinematichthys</i>	<i>Dinematichthys</i> spp.	Marine
Bythitidae		<i>Diplacanthopoma</i>	<i>Diplacanthopoma</i> spp.	Marine
Bythitidae		<i>Dipulus</i>	<i>Dipulus</i> spp.	Marine
Bythitidae		<i>Eusurculus</i>	<i>Eusurculus</i> spp.	Marine
Bythitidae		<i>Fiordichthys</i>	<i>Fiordichthys</i> spp.	Marine
Bythitidae		<i>Grammonus</i>	<i>Grammonus</i> spp.	Marine
Bythitidae		<i>Gunterichthys</i>	<i>Gunterichthys</i> spp.	Marine
Bythitidae		<i>Hastatobythites</i>	<i>Hastatobythites</i> spp.	Marine
Bythitidae		<i>Hepthocara</i>	<i>Hepthocara</i> spp.	Marine
Bythitidae		<i>Lapitaichthys</i>	<i>Lapitaichthys</i> spp.	Marine
Bythitidae		<i>Lucifuga</i>	<i>Lucifuga</i> spp.	Marine
Bythitidae		<i>Majungaichthys</i>	<i>Majungaichthys</i> spp.	Marine
Bythitidae		<i>Mascarenichthys</i>	<i>Mascarenichthys</i> spp.	Marine
Bythitidae		<i>Melodichthys</i>	<i>Melodichthys</i> spp.	Marine
Bythitidae		<i>Microbrotula</i>	<i>Microbrotula</i> spp.	Marine
Bythitidae		<i>Monothrix</i>	<i>Monothrix</i> spp.	Marine
Bythitidae		<i>Nielsenichthys</i>	<i>Nielsenichthys</i> spp.	Marine
Bythitidae		<i>Ogilbia</i>	<i>Ogilbia</i> spp.	Marine
Bythitidae		<i>Ogilbichthys</i>	<i>Ogilbichthys</i> spp.	Marine
Bythitidae		<i>Paradiancistrus</i>	<i>Paradiancistrus</i> spp.	Marine
Bythitidae		<i>Porocephalichthys</i>	<i>Porocephalichthys</i> spp.	Marine
Bythitidae		<i>Pseudogilbia</i>	<i>Pseudogilbia</i> spp.	Marine
Bythitidae		<i>Pseudonus</i>	<i>Pseudonus</i> spp.	Marine
Bythitidae		<i>Saccogaster</i>	<i>Saccogaster</i> spp.	Marine

Bythitidae		<i>Stygnobrotula</i>	<i>Stygnobrotula</i> spp.	Marine
Bythitidae		<i>Thalassobathia</i>	<i>Thalassobathia</i> spp.	Marine
Bythitidae		<i>Thermichthys</i>	<i>Thermichthys</i> spp.	Marine
Bythitidae		<i>Timorichthys</i>	<i>Timorichthys</i> spp.	Marine
Bythitidae		<i>Tuamotuichthys</i>	<i>Tuamotuichthys</i> spp.	Marine
Bythitidae		<i>Typhliasina</i>	<i>Typhliasina</i> spp.	Marine
Bythitidae		<i>Ungusurculus</i>	<i>Ungusurculus</i> spp.	Marine
Bythitidae		<i>Zephyrichthys</i>	<i>Zephyrichthys</i> spp.	Marine
Callichthyidae	Callichthyinae	<i>Dianema</i>	<i>Dianema urostriatum</i>	Other Freshwater
Callichthyidae	Corydoradinae	<i>Brochis</i>	<i>Brochis</i> spp.	Other Freshwater
Callichthyidae		<i>Corydoras</i>	<i>Corydoras</i> spp.	Other Freshwater
Callionymidae		<i>Anaora</i>	<i>Anaora</i> spp.	Marine
Callionymidae		<i>Bathycallionymus</i>	<i>Bathycallionymus</i> spp.	Marine
Callionymidae		<i>Callionymus</i>	<i>Callionymus</i> spp.	Marine
Callionymidae		<i>Calliurichthys</i>	<i>Calliurichthys</i> spp.	Marine
Callionymidae		<i>Dactylopus</i>	<i>Dactylopus</i> spp.	Marine
Callionymidae		<i>Diplogrammus</i>	<i>Diplogrammus</i> spp.	Marine
Callionymidae		<i>Draculo</i>	<i>Draculo</i> spp.	Marine
Callionymidae		<i>Eleutherochir</i>	<i>Eleutherochir</i> spp.	Marine
Callionymidae		<i>Eocallionymus</i>	<i>Eocallionymus</i> spp.	Marine
Callionymidae		<i>Foetorepus</i>	<i>Foetorepus</i> spp.	Marine
Callionymidae		<i>Minysynchiropus</i>	<i>Minysynchiropus</i> spp.	Marine
Callionymidae		<i>Neosynchiropus</i>	<i>Neosynchiropus</i> spp.	Marine
Callionymidae		<i>Paracallionymus</i>	<i>Paracallionymus</i> spp.	Marine
Callionymidae		<i>Protogrammus</i>	<i>Protogrammus</i> spp.	Marine
Callionymidae		<i>Pseudocalliurichthys</i>	<i>Pseudocalliurichthys</i> spp.	Marine
Callionymidae		<i>Repomucenus</i>	<i>Repomucenus</i> spp.	Marine
Callionymidae		<i>Synchiropus</i>	<i>Synchiropus</i> spp.	Marine
Callionymidae		<i>Tonlespia</i>	<i>Tonlespia</i> spp.	Marine
Carangidae		<i>Alectis</i>	<i>Alectis</i> spp.	Marine
Carapidae		<i>Carapus</i>	<i>Carapus</i> spp.	Marine
Carapidae		<i>Echiodon</i>	<i>Echiodon</i> spp.	Marine
Carapidae		<i>Encheliophis</i>	<i>Encheliophis</i> spp.	Marine
Carapidae		<i>Eurypleuron</i>	<i>Eurypleuron</i> spp.	Marine
Carapidae		<i>Onuxodon</i>	<i>Onuxodon</i> spp.	Marine
Carapidae		<i>Pyramodon</i>	<i>Pyramodon</i> spp.	Marine
Carapidae		<i>Snyderidia</i>	<i>Snyderidia</i> spp.	Marine
Carapidae		<i>Tetragondacnus</i>	<i>Tetragondacnus</i> spp.	Marine
Centriscidae		<i>Aeoliscus</i>	<i>Aeoliscus</i> spp.	Marine
Centriscidae		<i>Centriscops</i>	<i>Centriscops</i> spp.	Marine
Centriscidae		<i>Centriscus</i>	<i>Centriscus</i> spp.	Marine
Centriscidae		<i>Macroramphosus</i>	<i>Macroramphosus</i> spp.	Marine
Centriscidae		<i>Notopogon</i>	<i>Notopogon</i> spp.	Marine
Chaetodontidae		<i>Amphichaetodon</i>	<i>Amphichaetodon</i> spp.	Marine
Chaetodontidae		<i>Chaetodon</i>	<i>Chaetodon</i> spp.	Marine
Chaetodontidae		<i>Chelmon</i>	<i>Chelmon</i> spp.	Marine

Chaetodontidae		<i>Chelmonops</i>	<i>Chelmonops</i> spp.	Marine
Chaetodontidae		<i>Coradion</i>	<i>Coradion</i> spp.	Marine
Chaetodontidae		<i>Forcipiger</i>	<i>Forcipiger</i> spp.	Marine
Chaetodontidae		<i>Hemitaurichthys</i>	<i>Hemitaurichthys</i> spp.	Marine
Chaetodontidae		<i>Heniochus</i>	<i>Heniochus</i> spp.	Marine
Chaetodontidae		<i>Johnrandallia</i>	<i>Johnrandallia</i> spp.	Marine
Chaetodontidae		<i>Parachaetodon</i>	<i>Parachaetodon</i> spp.	Marine
Chaetodontidae		<i>Prognathodes</i>	<i>Prognathodes</i> spp.	Marine
Chaetodontidae		<i>Roa</i>	<i>Roa</i> spp.	Marine
Characidae	Aphyocharacinae	<i>Aphyocharax</i>	<i>Aphyocharax</i> spp.	Other Freshwater
Characidae	Glandulocaudinae	<i>Corynopoma</i>	<i>Corynopoma riisei</i>	Other Freshwater
Characidae	Glandulocaudinae	<i>Mimagoniates</i>	<i>Mimagoniates microlepis</i>	Other Freshwater
Characidae	Serrasalminae	<i>Metynnis</i>	<i>Metynnis</i> spp.	Other Freshwater
Characidae	Serrasalminae	<i>Myloplus</i>	<i>Myloplus rubripinnis</i>	Other Freshwater
Characidae		<i>Astyanax</i>	<i>Astyanax jordani</i>	Other Freshwater
Characidae		<i>Boehlkea</i>	<i>Boehlkea fredcochui</i>	Other Freshwater
Characidae		<i>Gymnocorymbus</i>	<i>Gymnocorymbus ternetzi</i>	Other Freshwater
Characidae		<i>Hasemanina</i>	<i>Hasemanina nana</i>	Other Freshwater
Characidae		<i>Hemigrammus</i>	<i>Hemigrammus</i> spp.	Other Freshwater
Characidae		<i>Hyphessobrycon</i>	<i>Hyphessobrycon</i> spp.	Other Freshwater
Characidae		<i>Hyphessobrycon</i>	<i>Megalampodus</i> spp.	Other Freshwater
Characidae		<i>Inpaichthys</i>	<i>Inpaichthys kerri</i>	Other Freshwater
Characidae		<i>Moenkhausia</i>	<i>Moenkhausia</i> spp.	Other Freshwater
Characidae		<i>Nematobrycon</i>	<i>Nematobrycon</i> spp.	Other Freshwater
Characidae		<i>Paracheirodon</i>	<i>Paracheirodon axelrodi</i>	Other Freshwater
Characidae		<i>Paracheirodon</i>	<i>Paracheirodon innesi</i>	Other Freshwater
Characidae		<i>Petitella</i>	<i>Petitella georgiae</i>	Other Freshwater
Characidae		<i>Prionobrama</i>	<i>Prionobrama filigera</i>	Other Freshwater
Characidae		<i>Pristella</i>	<i>Pristella maxillaris</i>	Other Freshwater
Characidae		<i>Thayeria</i>	<i>Thayeria</i> spp.	Other Freshwater
Characidae		<i>Triportheus</i>	<i>Triportheus</i> spp.	Other Freshwater
Chilodontidae		<i>Chilodus</i>	<i>Chilodus punctatus</i>	Other Freshwater
Cichlidae		<i>Aequidens</i>	<i>Aequidens pulcher</i>	Cichlid
Cichlidae		<i>Apistogramma</i>	<i>Apistogramma</i> spp.	Cichlid
Cichlidae		<i>Astronotus</i>	<i>Astronotus ocellatus</i>	Cichlid
Cichlidae		<i>Aulonocara</i>	<i>Aulonocara</i> spp.	Cichlid
Cichlidae		<i>Aulonocara</i>	<i>Aulonocara nyassae</i>	Cichlid
Cichlidae		<i>Benthochromis</i>	<i>Benthochromis tricot</i>	Cichlid
Cichlidae		<i>Chalinochromis</i>	<i>Chalinochromis</i> spp.	Cichlid
Cichlidae		<i>Chalinochromis</i>	<i>Chalinochromis brichardi</i>	Cichlid
Cichlidae		<i>Chilotilapia</i>	<i>Chilotilapia rhoadesii</i>	Cichlid
Cichlidae		<i>Cleithracara</i>	<i>Cleithracara maronii</i>	Cichlid
Cichlidae		<i>Cyathopharynx</i>	<i>Cyathopharynx furcifer</i>	Cichlid
Cichlidae		<i>Cyphotilapia</i>	<i>Cyphotilapia frontosa</i>	Cichlid
Cichlidae		<i>Cyprichromis</i>	<i>Cyprichromis leptosoma</i>	Cichlid
Cichlidae		<i>Cyrtocara</i>	<i>Cyrtocara moorii</i>	Cichlid

Cichlidae	<i>Dicrossus</i>	<i>Dicrossus filamentosus</i>	Cichlid
Cichlidae	<i>Dicrossus</i>	<i>Dicrossus maculatus</i>	Cichlid
Cichlidae	<i>Eretmodus</i>	<i>Eretmodus cyanostictus</i>	Cichlid
Cichlidae	<i>Gnathochromis</i>	<i>Gnathochromis permaxillaris</i>	Cichlid
Cichlidae	<i>Hypsophrys</i>	<i>Hypsophrys nicaraguense</i>	Cichlid
Cichlidae	<i>Iodotropheus</i>	<i>Iodotropheus sprengerae</i>	Cichlid
Cichlidae	<i>Julidochromis</i>	<i>Julidochromis spp.</i>	Cichlid
Cichlidae	<i>Laetacara</i>	<i>Laetacara curviceps</i>	Cichlid
Cichlidae	<i>Laetacara</i>	<i>Laetacara dorsigera</i>	Cichlid
Cichlidae	<i>Lamprologus</i>	<i>Lamprologus ocellatus</i>	Cichlid
Cichlidae	<i>Melanochromis</i>	<i>Melanochromis auratus</i>	Cichlid
Cichlidae	<i>Melanochromis</i>	<i>Melanochromis simulans</i>	Cichlid
Cichlidae	<i>Mesonauta</i>	<i>Mesonauta festivus</i>	Cichlid
Cichlidae	<i>Mikrogeophagus</i>	<i>Mikrogeophagus altispinosus</i>	Cichlid
Cichlidae	<i>Mikrogeophagus</i>	<i>Mikrogeophagus ramirezi</i>	Cichlid
Cichlidae	<i>Nannacara</i>	<i>Nannacara anomala</i>	Cichlid
Cichlidae	<i>Nannacara</i>	<i>Nannacara aureocephalus</i>	Cichlid
Cichlidae	<i>Nannacara</i>	<i>Nannacara taenia</i>	Cichlid
Cichlidae	<i>Neolamprologus</i>	<i>Neolamprologus brichardi</i>	Cichlid
Cichlidae	<i>Neolamprologus</i>	<i>Neolamprologus cylindricus</i>	Cichlid
Cichlidae	<i>Neolamprologus</i>	<i>Neolamprologus leleupi</i>	Cichlid
Cichlidae	<i>Neolamprologus</i>	<i>Neolamprologus meeli</i>	Cichlid
Cichlidae	<i>Neolamprologus</i>	<i>Neolamprologus mustax</i>	Cichlid
Cichlidae	<i>Ophthalmotilapia</i>	<i>Ophthalmotilapia spp.</i>	Cichlid
Cichlidae	<i>Paracyprichromis</i>	<i>Paracyprichromis nigripinnis</i>	Cichlid
Cichlidae	<i>Pelvicachromis</i>	<i>Pelvicachromis pulcher</i>	Cichlid
Cichlidae	<i>Pelvicachromis</i>	<i>Pelvicachromis subocellatus</i>	Cichlid
Cichlidae	<i>Pelvicachromis</i>	<i>Pelvicachromis taeniatus</i>	Cichlid
Cichlidae	<i>Petrochromis</i>	<i>Petrochromis trewavasae trew</i>	Cichlid
Cichlidae	<i>Pterophyllum</i>	<i>Pterophyllum spp.</i>	Cichlid
Cichlidae	<i>Spathodus</i>	<i>Spathodus erythrodon</i>	Cichlid
Cichlidae	<i>Symphysodon</i>	<i>Symphysodon spp.</i>	Cichlid
Cichlidae	<i>Tanganicodus</i>	<i>Tanganicodus irsacae</i>	Cichlid
Cichlidae	<i>Tropheus</i>	<i>Tropheus spp.</i>	Cichlid
Cirrhitidae	<i>Amblycirrhitus</i>	<i>Amblycirrhitus spp.</i>	Marine
Cirrhitidae	<i>Cirrhitichthys</i>	<i>Cirrhitichthys spp.</i>	Marine
Cirrhitidae	<i>Cirrhitops</i>	<i>Cirrhitops spp.</i>	Marine
Cirrhitidae	<i>Cirrhitus</i>	<i>Cirrhitus spp.</i>	Marine
Cirrhitidae	<i>Cristacirrhitus</i>	<i>Cristacirrhitus spp.</i>	Marine
Cirrhitidae	<i>Cyprinocirrhitus</i>	<i>Cyprinocirrhitus spp.</i>	Marine
Cirrhitidae	<i>Isocirrhitus</i>	<i>Isocirrhitus spp.</i>	Marine
Cirrhitidae	<i>Itycirrhitus</i>	<i>Itycirrhitus spp.</i>	Marine
Cirrhitidae	<i>Neocirrhitus</i>	<i>Neocirrhitus spp.</i>	Marine
Cirrhitidae	<i>Notocirrhitus</i>	<i>Notocirrhitus spp.</i>	Marine
Cirrhitidae	<i>Oxycirrhitus</i>	<i>Oxycirrhitus spp.</i>	Marine
Cirrhitidae	<i>Paracirrhitus</i>	<i>Paracirrhitus spp.</i>	Marine

Cobitidae	Botiinae	<i>Botia</i>	<i>Botia lohachata</i>	Other Freshwater
Cobitidae	Botiinae	<i>Chromobotia</i>	<i>Chromobotia macracanthus</i>	Other Freshwater
Cobitidae	Botiinae	<i>Yasuhikotakia</i>	<i>Yasuhikotakia sidthimunki</i>	Other Freshwater
Cobitidae		<i>Acanthopphthalmu</i>	<i>Acanthopphthalmus</i> spp.	Other Freshwater
Crenuchidae	Crenuchinae	<i>Poecilocharax</i>	<i>Poecilocharax weitzmani</i>	Other Freshwater
Cyprinidae		<i>Balantiocheilos</i>	<i>Balantiocheilos melanopterus</i>	Goldfish
Cyprinidae		<i>Boraras</i>	<i>Boraras maculates</i>	Goldfish
Cyprinidae		<i>Carassius</i>	<i>Carassius auratus auratus</i>	Goldfish
Cyprinidae		<i>Crossocheilus</i>	<i>Crossocheilus siamensis</i>	Other Freshwater
Cyprinidae		<i>Danio</i>	<i>Danio albolineatus</i>	Goldfish
Cyprinidae		<i>Danio</i>	<i>Danio kerri</i>	Goldfish
Cyprinidae		<i>Danio</i>	<i>Danio nigrofasciatus</i>	Goldfish
Cyprinidae		<i>Danio</i>	<i>Danio rerio</i>	Goldfish
Cyprinidae		<i>Devario</i>	<i>Devario devario</i>	Goldfish
Cyprinidae		<i>Devario</i>	<i>Devario malabaricus</i>	Goldfish
Cyprinidae		<i>Epalzeorhynchos</i>	<i>Epalzeorhynchos bicolor</i>	Goldfish
Cyprinidae		<i>Epalzeorhynchos</i>	<i>Epalzeorhynchos frenatum</i>	Goldfish
Cyprinidae		<i>Epalzeorhynchos</i>	<i>Epalzeorhynchos kalopterus</i>	Goldfish
Cyprinidae		<i>Epalzeorhynchos</i>	<i>Epalzeorhynchos munense</i>	Goldfish
Cyprinidae		<i>Esomus</i>	<i>Esomus malayensis</i>	Goldfish
Cyprinidae		<i>Labeo</i>	<i>Labeo chrysophekadion</i>	Goldfish
Cyprinidae		<i>Labeo</i>	<i>Labeo cyclorhynchus</i>	Goldfish
Cyprinidae		<i>Laubuca</i>	<i>Laubuca laubuca</i>	Other Freshwater
Cyprinidae		<i>Osteochilus</i>	<i>Osteochilus hasseltii</i>	Goldfish
Cyprinidae		<i>Osteochilus</i>	<i>Osteochilus vittatus</i>	Goldfish
Cyprinidae		<i>Parachela</i>	<i>Parachela oxygastroides</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius arulius</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius asoka</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius bimaculatus</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius conchonius</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius cumingii</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius everetti</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius fasciatus</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius filamentosus</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius hexazona</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius lateristriga</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius lineatus</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius nigrofasciatus</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius oligolepis</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius partipentazona</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius pentazona</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius tetrazona</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius ticto</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius tittैया</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius vittatus</i>	Goldfish
Cyprinidae		<i>Puntius</i>	<i>Puntius semifasciolatus</i>	Goldfish

Cyprinidae		<i>Rasbora</i>	<i>Rasbora argyrotaenia</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora borapetensis</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora caudimaculata</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora dorsiocellata</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora dusonensis</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora einthovenii</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora elegans</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora kalochroma</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora leptosome</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora pauciperforata</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora sarawakensis</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora steineri</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora trilineata</i>	Goldfish
Cyprinidae		<i>Rasbora</i>	<i>Rasbora vaterifloris</i>	Goldfish
Cyprinidae		<i>Rhodeus</i>	<i>Rhodeus amarus</i>	Goldfish
Cyprinidae		<i>Rhodeus</i>	<i>Rhodeus sericeus</i>	Goldfish
Cyprinidae		<i>Sawbwa</i>	<i>Sawbwa resplendens</i>	Goldfish
Cyprinidae		<i>Tanichthys</i>	<i>Tanichthys albonubes</i>	Goldfish
Cyprinidae		<i>Trigonostigma</i>	<i>Trigonostigma heteromorpha</i>	Goldfish
Cyprinidae		<i>Trigonostigma</i>	<i>Trigonostigma hengeli</i>	Goldfish
Dasyatidae		<i>Taeniura</i>	<i>Taeniura lymma</i>	Marine
Eleotridae	Eleotrinae	<i>Tateurndina</i>	<i>Tateurndina ocellicauda</i>	Other Freshwater
Ephippidae		<i>Chaetodipterus</i>	<i>Chaetodipterus</i> spp.	Marine
Ephippidae		<i>Ephippus</i>	<i>Ephippus</i> spp.	Marine
Ephippidae		<i>Parapsettus</i>	<i>Parapsettus</i> spp.	Marine
Ephippidae		<i>Platax</i>	<i>Platax</i> spp.	Marine
Ephippidae		<i>Proteracanthus</i>	<i>Proteracanthus</i> spp.	Marine
Ephippidae		<i>Rhinoprenes</i>	<i>Rhinoprenes</i> spp.	Marine
Ephippidae		<i>Tripterodon</i>	<i>Tripterodon</i> spp.	Marine
Ephippidae		<i>Zabidius</i>	<i>Zabidius</i> spp.	Marine
Gasteropelecidae		<i>Carnegiella</i>	<i>Carnegiella</i> spp.	Other Freshwater
Gasteropelecidae		<i>Gasteropelecus</i>	<i>Gasteropelecus</i> spp.	Other Freshwater
Gasteropelecidae		<i>Thoracocharax</i>	<i>Thoracocharax</i> spp.	Other Freshwater
Gobiidae	Gobiinae	<i>Gobiodon</i>	<i>Gobiodon</i> spp.	Marine
Gobiidae	Gobiinae	<i>Lythrypnus</i>	<i>Lythrypnus</i> spp.	Marine
Gobiidae	Gobiinae	<i>Signigobius</i>	<i>Signigobius</i> spp.	Marine
Gobiidae	Gobiinae	<i>Valenciennesa</i>	<i>Valenciennesa strigata</i>	Marine
Gobiidae	Gobionellinae	<i>Brachygobius</i>	<i>Brachygobius</i> spp.	Other Freshwater
Grammatidae		<i>Gamma</i>	<i>Gamma</i> spp.	Marine
Grammatidae		<i>Lipogramma</i>	<i>Lipogramma</i> spp.	Marine
Gyrinocheilidae		<i>Gyrinocheilus</i>	<i>Gyrinocheilus aymonieri</i>	Other Freshwater
Hemiodontidae		<i>Hemiodus</i>	<i>Hemiodus sterni</i>	Other Freshwater
Heterodontidae		<i>Heterodontus</i>	<i>Heterodontus zebra</i>	Marine
Holocentridae		<i>Corniger</i>	<i>Corniger</i> spp.	Marine
Holocentridae		<i>Holocentrus</i>	<i>Holocentrus</i> spp.	Marine
Holocentridae		<i>Myripristis</i>	<i>Myripristis</i> spp.	Marine

Holocentridae	<i>Neoniphon</i>	<i>Neoniphon</i> spp.	Marine
Holocentridae	<i>Ostichthys</i>	<i>Ostichthys</i> spp.	Marine
Holocentridae	<i>Plectrypops</i>	<i>Plectrypops</i> spp.	Marine
Holocentridae	<i>Pristilepis</i>	<i>Pristilepis</i> spp.	Marine
Holocentridae	<i>Sargocentron</i>	<i>Sargocentron</i> spp.	Marine
Labridae	<i>Acantholabrus</i>	<i>Acantholabrus</i> spp.	Marine
Labridae	<i>Achoerodus</i>	<i>Achoerodus</i> spp.	Marine
Labridae	<i>Ammolabrus</i>	<i>Ammolabrus</i> spp.	Marine
Labridae	<i>Anampses</i>	<i>Anampses</i> spp.	Marine
Labridae	<i>Anchichoerops</i>	<i>Anchichoerops</i> spp.	Marine
Labridae	<i>Austrolabrus</i>	<i>Austrolabrus</i> spp.	Marine
Labridae	<i>Bodianus</i>	<i>Bodianus</i> spp.	Marine
Labridae	<i>Centrolabrus</i>	<i>Centrolabrus</i> spp.	Marine
Labridae	<i>Cheilinus</i>	<i>Cheilinus</i> spp.	Marine
Labridae	<i>Cheilio</i>	<i>Cheilio</i> spp.	Marine
Labridae	<i>Choerodon</i>	<i>Choerodon</i> spp.	Marine
Labridae	<i>Cirrhilabrus</i>	<i>Cirrhilabrus</i> spp.	Marine
Labridae	<i>Clepticus</i>	<i>Clepticus</i> spp.	Marine
Labridae	<i>Conniella</i>	<i>Conniella</i> spp.	Marine
Labridae	<i>Coris</i>	<i>Coris</i> spp.	Marine
Labridae	<i>Ctenolabrus</i>	<i>Ctenolabrus</i> spp.	Marine
Labridae	<i>Cymolutes</i>	<i>Cymolutes</i> spp.	Marine
Labridae	<i>Decodon</i>	<i>Decodon</i> spp.	Marine
Labridae	<i>Diproctacanthus</i>	<i>Diproctacanthus</i> spp.	Marine
Labridae	<i>Doratonotus</i>	<i>Doratonotus</i> spp.	Marine
Labridae	<i>Dotalabrus</i>	<i>Dotalabrus</i> spp.	Marine
Labridae	<i>Epibulus</i>	<i>Epibulus</i> spp.	Marine
Labridae	<i>Eupetrichthys</i>	<i>Eupetrichthys</i> spp.	Marine
Labridae	<i>Frontilabrus</i>	<i>Frontilabrus</i> spp.	Marine
Labridae	<i>Gomphosus</i>	<i>Gomphosus</i> spp.	Marine
Labridae	<i>Halichoeres</i>	<i>Halichoeres</i> spp.	Marine
Labridae	<i>Hemigymnus</i>	<i>Hemigymnus</i> spp.	Marine
Labridae	<i>Hologymnosus</i>	<i>Hologymnosus</i> spp.	Marine
Labridae	<i>Iniistius</i>	<i>Iniistius</i> spp.	Marine
Labridae	<i>Labrichthys</i>	<i>Labrichthys</i> spp.	Marine
Labridae	<i>Labroides</i>	<i>Labroides</i> spp.	Marine
Labridae	<i>Labropsis</i>	<i>Labropsis</i> spp.	Marine
Labridae	<i>Labrus</i>	<i>Labrus</i> spp.	Marine
Labridae	<i>Lachnolaimus</i>	<i>Lachnolaimus</i> spp.	Marine
Labridae	<i>Lappanella</i>	<i>Lappanella</i> spp.	Marine
Labridae	<i>Larabicus</i>	<i>Larabicus</i> spp.	Marine
Labridae	<i>Leptojulius</i>	<i>Leptojulius</i> spp.	Marine
Labridae	<i>Macropharyngod</i>	<i>Macropharyngodon</i> spp.	Marine
Labridae	<i>Malapterus</i>	<i>Malapterus</i> spp.	Marine
Labridae	<i>Minilabrus</i>	<i>Minilabrus</i> spp.	Marine
Labridae	<i>Nelabrichthys</i>	<i>Nelabrichthys</i> spp.	Marine

Labridae		<i>Notolabrus</i>	<i>Notolabrus</i> spp.	Marine
Labridae		<i>Novaculichthys</i>	<i>Novaculichthys</i> spp.	Marine
Labridae		<i>Novaculoides</i>	<i>Novaculoides</i> spp.	Marine
Labridae		<i>Ophthalmolepis</i>	<i>Ophthalmolepis</i> spp.	Marine
Labridae		<i>Oxycheilinus</i>	<i>Oxycheilinus</i> spp.	Marine
Labridae		<i>Oxyjulis</i>	<i>Oxyjulis</i> spp.	Marine
Labridae		<i>Paracheilinus</i>	<i>Paracheilinus</i> spp.	Marine
Labridae		<i>Parajulis</i>	<i>Parajulis</i> spp.	Marine
Labridae		<i>Pictilabrus</i>	<i>Pictilabrus</i> spp.	Marine
Labridae		<i>Polylepion</i>	<i>Polylepion</i> spp.	Marine
Labridae		<i>Pseudocheilinops</i>	<i>Pseudocheilinops</i> spp.	Marine
Labridae		<i>Pseudocheilinus</i>	<i>Pseudocheilinus</i> spp.	Marine
Labridae		<i>Pseudocoris</i>	<i>Pseudocoris</i> spp.	Marine
Labridae		<i>Pseudodax</i>	<i>Pseudodax</i> spp.	Marine
Labridae		<i>Pseudojuloides</i>	<i>Pseudojuloides</i> spp.	Marine
Labridae		<i>Pseudolabrus</i>	<i>Pseudolabrus</i> spp.	Marine
Labridae		<i>Pteragogus</i>	<i>Pteragogus</i> spp.	Marine
Labridae		<i>Semicossyphus</i>	<i>Semicossyphus</i> spp.	Marine
Labridae		<i>Stethojulis</i>	<i>Stethojulis</i> spp.	Marine
Labridae		<i>Suezichthys</i>	<i>Suezichthys</i> spp.	Marine
Labridae		<i>Symphodus</i>	<i>Symphodus</i> spp.	Marine
Labridae		<i>Tautoga</i>	<i>Tautoga</i> spp.	Marine
Labridae		<i>Tautogolabrus</i>	<i>Tautogolabrus</i> spp.	Marine
Labridae		<i>Terelabrus</i>	<i>Terelabrus</i> spp.	Marine
Labridae		<i>Thalassoma</i>	<i>Thalassoma</i> spp.	Marine
Labridae		<i>Wetmorella</i>	<i>Wetmorella</i> spp.	Marine
Labridae		<i>Xenajulis</i>	<i>Xenajulis</i> spp.	Marine
Labridae		<i>Xiphocheilus</i>	<i>Xiphocheilus</i> spp.	Marine
Labridae		<i>Xyrichtys</i>	<i>Xyrichtys</i> spp.	Marine
Lebiasinidae	Pyrrhulininae	<i>Copeina</i>	<i>Copeina guttata</i>	Other Freshwater
Lebiasinidae	Pyrrhulininae	<i>Copella</i>	<i>Copella arnoldi</i>	Other Freshwater
Lebiasinidae	Pyrrhulininae	<i>Nannostomus</i>	<i>Nannostomus</i> spp.	Other Freshwater
Lobotidae		<i>Lobotes</i>	<i>Lobotes</i> spp.	Marine
Loricariidae	Ancistrinae	<i>Dekeyseria</i>	<i>Dekeyseria pulchra</i>	Other Freshwater
Loricariidae	Hypoptopomatini	<i>Otocinclus</i>	<i>Otocinclus flexilis</i>	Other Freshwater
Loricariidae	Loricariinae	<i>Dasylicaria</i>	<i>Dasylicaria filamentosa</i>	Other Freshwater
Loricariidae	Loricariinae	<i>Farlowella</i>	<i>Farlowella acus</i>	Other Freshwater
Loricariidae	Loricariinae	<i>Sturisoma</i>	<i>Sturisoma panamense</i>	Other Freshwater
Lutjanidae	Lutjaninae	<i>Macolor</i>	<i>Macolor</i> spp.	Marine
Lutjanidae	Paradicichthyinae	<i>Symphorichthys</i>	<i>Symphorichthys</i> spp.	Marine
Malacanthidae		<i>Branchiostegus</i>	<i>Branchiostegus</i> spp.	Marine
Malacanthidae		<i>Caulolatilus</i>	<i>Caulolatilus</i> spp.	Marine
Malacanthidae		<i>Hoplolatilus</i>	<i>Hoplolatilus</i> spp.	Marine
Malacanthidae		<i>Lopholatilus</i>	<i>Lopholatilus</i> spp.	Marine
Malacanthidae		<i>Malacanthus</i>	<i>Malacanthus</i> spp.	Marine
Mastacembelidae		<i>Macrogathus</i>	<i>Macrogathus aculeatus</i>	Other Freshwater

Melanotaeniidae	<i>Glossolepis</i>	<i>Glossolepis incisa</i>	Other Freshwater
Mochokidae	<i>Synodontis</i>	<i>Synodontis decora</i>	Other Freshwater
Mochokidae	<i>Synodontis</i>	<i>Synodontis multipunctata</i>	Other Freshwater
Mochokidae	<i>Synodontis</i>	<i>Synodontis nigriventris</i>	Other Freshwater
Monacanthidae	<i>Acanthaluteres</i>	<i>Acanthaluteres</i> spp.	Marine
Monacanthidae	<i>Acreichthys</i>	<i>Acreichthys</i> spp.	Marine
Monacanthidae	<i>Aluterus</i>	<i>Aluterus</i> spp.	Marine
Monacanthidae	<i>Amanses</i>	<i>Amanses</i> spp.	Marine
Monacanthidae	<i>Anacanthus</i>	<i>Anacanthus</i> spp.	Marine
Monacanthidae	<i>Brachaluteres</i>	<i>Brachaluteres</i> spp.	Marine
Monacanthidae	<i>Cantherhines</i>	<i>Cantherhines</i> spp.	Marine
Monacanthidae	<i>Cantheschenia</i>	<i>Cantheschenia</i> spp.	Marine
Monacanthidae	<i>Chaetodermis</i>	<i>Chaetodermis</i> spp.	Marine
Monacanthidae	<i>Colurodontis</i>	<i>Colurodontis</i> spp.	Marine
Monacanthidae	<i>Enigmacanthus</i>	<i>Enigmacanthus</i> spp.	Marine
Monacanthidae	<i>Eubalichthys</i>	<i>Eubalichthys</i> spp.	Marine
Monacanthidae	<i>Lalmohania</i>	<i>Lalmohania</i> spp.	Marine
Monacanthidae	<i>Meuschenia</i>	<i>Meuschenia</i> spp.	Marine
Monacanthidae	<i>Monacanthus</i>	<i>Monacanthus</i> spp.	Marine
Monacanthidae	<i>Nelusetta</i>	<i>Nelusetta</i> spp.	Marine
Monacanthidae	<i>Oxymonacanthus</i>	<i>Oxymonacanthus</i> spp.	Marine
Monacanthidae	<i>Paraluteres</i>	<i>Paraluteres</i> spp.	Marine
Monacanthidae	<i>Paramonacanthus</i>	<i>Paramonacanthus</i> spp.	Marine
Monacanthidae	<i>Pervagor</i>	<i>Pervagor</i> spp.	Marine
Monacanthidae	<i>Pseudalutarius</i>	<i>Pseudalutarius</i> spp.	Marine
Monacanthidae	<i>Pseudomonacanth</i>	<i>Pseudomonacanthus</i> spp.	Marine
Monacanthidae	<i>Rudarius</i>	<i>Rudarius</i> spp.	Marine
Monacanthidae	<i>Scobinichthys</i>	<i>Scobinichthys</i> spp.	Marine
Monacanthidae	<i>Stephanolepis</i>	<i>Stephanolepis</i> spp.	Marine
Monacanthidae	<i>Thamnaconus</i>	<i>Thamnaconus</i> spp.	Marine
Monocentridae	<i>Cleidopus</i>	<i>Cleidopus</i> spp.	Marine
Monocentridae	<i>Monocentris</i>	<i>Monocentris</i> spp.	Marine
Mormyridae	<i>Campylomormyru</i>	<i>Campylomormyrus cassaicus</i>	Other Freshwater
Mormyridae	<i>Campylomormyru</i>	<i>Campylomormyrus rhynchoph</i>	Other Freshwater
Mormyridae	<i>Gnathonemus</i>	<i>Gnathonemus petersii</i>	Other Freshwater
Mormyridae	<i>Marcusenius</i>	<i>Marcusenius macrolepidotus</i>	Other Freshwater
Mullidae	<i>Mulloidichthys</i>	<i>Mulloidichthys</i> spp.	Marine
Mullidae	<i>Mullus</i>	<i>Mullus</i> spp.	Marine
Mullidae	<i>Parupeneus</i>	<i>Parupeneus</i> spp.	Marine
Mullidae	<i>Pseudupeneus</i>	<i>Pseudupeneus</i> spp.	Marine
Mullidae	<i>Upeneichthys</i>	<i>Upeneichthys</i> spp.	Marine
Mullidae	<i>Upeneus</i>	<i>Upeneus</i> spp.	Marine
Muraenidae	<i>Anarchias</i>	<i>Anarchias</i> spp.	Marine
Muraenidae	<i>Channomuraena</i>	<i>Channomuraena</i> spp.	Marine
Muraenidae	<i>Cirrimaxilla</i>	<i>Cirrimaxilla</i> spp.	Marine
Muraenidae	<i>Diaphenchelys</i>	<i>Diaphenchelys</i> spp.	Marine

Muraenidae		<i>Echidna</i>	<i>Echidna</i> spp.	Marine
Muraenidae		<i>Enchelycore</i>	<i>Enchelycore</i> spp.	Marine
Muraenidae		<i>Enchelynassa</i>	<i>Enchelynassa</i> spp.	Marine
Muraenidae		<i>Gymnomuraena</i>	<i>Gymnomuraena</i> spp.	Marine
Muraenidae		<i>Gymnothorax</i>	<i>Gymnothorax</i> spp.	Marine
Muraenidae		<i>Monopenchelys</i>	<i>Monopenchelys</i> spp.	Marine
Muraenidae		<i>Muraena</i>	<i>Muraena</i> spp.	Marine
Muraenidae		<i>Psedechidna</i>	<i>Psedechidna</i> spp.	Marine
Muraenidae		<i>Rhinomuraena</i>	<i>Rhinomuraena</i> spp.	Marine
Muraenidae		<i>Scuticaria</i>	<i>Scuticaria</i> spp.	Marine
Muraenidae		<i>Strophidon</i>	<i>Strophidon</i> spp.	Marine
Muraenidae		<i>Uropterygius</i>	<i>Uropterygius</i> spp.	Marine
Nemipter		<i>Scolopsis</i>	<i>Scolopsis bilineata</i>	Marine
Nothobranchiidae		<i>Aphyosemion</i>	<i>Aphyosemion</i> spp.	Other Freshwater
Nothobranchiidae		<i>Epiplatys</i>	<i>Epiplatys</i> spp.	Other Freshwater
Osphronemidae	Macropodusinae	<i>Macropodus</i>	<i>Macropodus opercularis</i>	Gourami
Osphronemidae	Macropodusinae	<i>Parosphromenus</i>	<i>Parosphromenus deissneri</i>	Gourami
Osphronemidae	Macropodusinae	<i>Betta</i>	<i>Betta</i> spp.	Gourami
Osphronemidae	Luciocephalinae	<i>Colisa</i>	<i>Colisa chuna</i>	Gourami
Osphronemidae	Luciocephalinae	<i>Colisa</i>	<i>Colisa fasciata</i>	Gourami
Osphronemidae	Luciocephalinae	<i>Colisa</i>	<i>Colisa labiosa</i>	Gourami
Osphronemidae	Luciocephalinae	<i>Colisa</i>	<i>Colisa lalia</i>	Gourami
Helostomatidae		<i>Helostoma</i>	<i>Helostoma temminkii</i>	Other Freshwater
Osphronemidae	Luciocephalinae	<i>Sphaerichthys</i>	<i>Sphaerichthys osphromenoides</i>	Gourami
Osphronemidae	Luciocephalinae	<i>Trichogaster</i>	<i>Trichogaster leeri</i>	Gourami
Osphronemidae	Luciocephalinae	<i>Trichogaster</i>	<i>Trichogaster microlepis</i>	Gourami
Osphronemidae	Luciocephalinae	<i>Trichogaster</i>	<i>Trichogaster trichopterus</i>	Gourami
Osphronemidae	Macropodusinae	<i>Trichopsis</i>	<i>Trichopsis pumila</i>	Gourami
Osphronemidae	Macropodusinae	<i>Trichopsis</i>	<i>Trichopsis vittata</i>	Gourami
Ostraciidae		<i>Acanthostracion</i>	<i>Acanthostracion</i> spp.	Marine
Ostraciidae		<i>Lactophrys</i>	<i>Lactophrys</i> spp.	Marine
Ostraciidae		<i>Lactoria</i>	<i>Lactoria</i> spp.	Marine
Ostraciidae		<i>Ostracion</i>	<i>Ostracion</i> spp.	Marine
Ostraciidae		<i>Paracanthostracion</i>	<i>Paracanthostracion</i> spp.	Marine
Ostraciidae		<i>Rhinesomus</i>	<i>Rhinesomus</i> spp.	Marine
Ostraciidae		<i>Rhynchostracion</i>	<i>Rhynchostracion</i> spp.	Marine
Ostraciidae		<i>Tetrosomus</i>	<i>Tetrosomus</i> spp.	Marine
Pantodontidae		<i>Pantodon</i>	<i>Pantodon buchholzi</i>	Other Freshwater
Pegasidae		<i>Eurypegasus</i>	<i>Eurypegasus</i> spp.	Marine
Pegasidae		<i>Pegasus</i>	<i>Pegasus</i> spp.	Marine
Pempheridae		<i>Parapriacanthus</i>	<i>Parapriacanthus</i> spp.	Marine
Pempheridae		<i>Pempheris</i>	<i>Pempheris</i> spp.	Marine
Pholidichthyidae		<i>Pholidichthys</i>	<i>Pholidichthys</i> spp.	Marine
Pimelodidae		<i>Pimelodus</i>	<i>Pimelodus ornatus</i>	Other Freshwater
Pimelodidae		<i>Pimelodus</i>	<i>Pimelodus pictus</i>	Other Freshwater
Pinguipedidae		<i>Kochichthys</i>	<i>Kochichthys</i> spp.	Marine

Pinguipedidae	<i>Parapercis</i>	<i>Parapercis</i> spp.	Marine
Pinguipedidae	<i>Pinguipes</i>	<i>Pinguipes</i> spp.	Marine
Pinguipedidae	<i>Prolatilus</i>	<i>Prolatilus</i> spp.	Marine
Pinguipedidae	<i>Pseudopercis</i>	<i>Pseudopercis</i> spp.	Marine
Pinguipedidae	<i>Ryukyuperpis</i>	<i>Ryukyuperpis</i> spp.	Marine
Pinguipedidae	<i>Simiperpis</i>	<i>Simiperpis</i> spp.	Marine
Plesiopidae	<i>Acanthoclinus</i>	<i>Acanthoclinus</i> spp.	Marine
Plesiopidae	<i>Acanthoplesiops</i>	<i>Acanthoplesiops</i> spp.	Marine
Plesiopidae	<i>Assessor</i>	<i>Assessor</i> spp.	Marine
Plesiopidae	<i>Beliops</i>	<i>Beliops</i> spp.	Marine
Plesiopidae	<i>Belonepterygion</i>	<i>Belonepterygion</i> spp.	Marine
Plesiopidae	<i>Callopleiops</i>	<i>Callopleiops</i> spp.	Marine
Plesiopidae	<i>Fraudella</i>	<i>Fraudella</i> spp.	Marine
Plesiopidae	<i>Notograptus</i>	<i>Notograptus</i> spp.	Marine
Plesiopidae	<i>Paraplesiops</i>	<i>Paraplesiops</i> spp.	Marine
Plesiopidae	<i>Plesiops</i>	<i>Plesiops</i> spp.	Marine
Plesiopidae	<i>Steeneichthys</i>	<i>Steeneichthys</i> spp.	Marine
Plesiopidae	<i>Trachinops</i>	<i>Trachinops</i> spp.	Marine
Plotosidae	<i>Plotosus</i>	<i>Plotosus Inieatus</i>	Marine
Poeciliidae	<i>Poecilia</i>	<i>Poecilia latipinna</i>	Poecillid
Poeciliidae	<i>Poecilia</i>	<i>Poecilia reticulata</i>	Poecillid
Poeciliidae	<i>Poecilia</i>	<i>Poecilia sphenops</i>	Poecillid
Poeciliidae	<i>Poecilia</i>	<i>Poecilia velifera</i>	Poecillid
Poeciliidae	<i>Xiphophorus</i>	<i>Xiphophorus hellerii</i>	Poecillid
Poeciliidae	<i>Xiphophorus</i>	<i>Xiphophorus maculatus</i>	Poecillid
Poeciliidae	<i>Xiphophorus</i>	<i>Xiphophorus variatus</i>	Poecillid
Pomacanthidae	<i>Apolemichthys</i>	<i>Apolemichthys</i> spp.	Marine
Pomacanthidae	<i>Centropyge</i>	<i>Centropyge</i> spp.	Marine
Pomacanthidae	<i>Chaetodontoplus</i>	<i>Chaetodontoplus</i> spp.	Marine
Pomacanthidae	<i>Genicanthus</i>	<i>Genicanthus</i> spp.	Marine
Pomacanthidae	<i>Holacanthus</i>	<i>Holacanthus</i> spp.	Marine
Pomacanthidae	<i>Pomacanthus</i>	<i>Pomacanthus</i> spp.	Marine
Pomacanthidae	<i>Pygoplites</i>	<i>Pygoplites</i> spp.	Marine
Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf</i> spp.	Marine
Pomacentridae	<i>Acanthochromis</i>	<i>Acanthochromis</i> spp.	Marine
Pomacentridae	<i>Altrichthys</i>	<i>Altrichthys</i> spp.	Marine
Pomacentridae	<i>Amblyglyphidodo</i>	<i>Amblyglyphidodon</i> spp.	Marine
Pomacentridae	<i>Amblypomacentru</i>	<i>Amblypomacentrus</i> spp.	Marine
Pomacentridae	<i>Amphiprion</i>	<i>Amphiprion</i> spp.	Marine
Pomacentridae	<i>Azurina</i>	<i>Azurina</i> spp.	Marine
Pomacentridae	<i>Cheiloprion</i>	<i>Cheiloprion</i> spp.	Marine
Pomacentridae	<i>Chromis</i>	<i>Chromis</i> spp.	Marine
Pomacentridae	<i>Chrysiptera</i>	<i>Chrysiptera</i> spp.	Marine
Pomacentridae	<i>Dascyllus</i>	<i>Dascyllus</i> spp.	Marine
Pomacentridae	<i>Dischistodus</i>	<i>Dischistodus</i> spp.	Marine
Pomacentridae	<i>Hemiglyphidodon</i>	<i>Hemiglyphidodon</i> spp.	Marine

Pomacentridae	<i>Hypsypops</i>	<i>Hypsypops</i> spp.	Marine
Pomacentridae	<i>Lepidozygus</i>	<i>Lepidozygus</i> spp.	Marine
Pomacentridae	<i>Mecaenichthys</i>	<i>Mecaenichthys</i> spp.	Marine
Pomacentridae	<i>Microspathodon</i>	<i>Microspathodon</i> spp.	Marine
Pomacentridae	<i>Neoglyphidodon</i>	<i>Neoglyphidodon</i> spp.	Marine
Pomacentridae	<i>Neopomacentrus</i>	<i>Neopomacentrus</i> spp.	Marine
Pomacentridae	<i>Nexilosus</i>	<i>Nexilosus</i> spp.	Marine
Pomacentridae	<i>Parma</i>	<i>Parma</i> spp.	Marine
Pomacentridae	<i>Plectroglyphidodo</i>	<i>Plectroglyphidodon</i> spp.	Marine
Pomacentridae	<i>Pomacentrus</i>	<i>Pomacentrus</i> spp.	Marine
Pomacentridae	<i>Pomachromis</i>	<i>Pomachromis</i> spp.	Marine
Pomacentridae	<i>Premnas</i>	<i>Premnas</i> spp.	Marine
Pomacentridae	<i>Pristotis</i>	<i>Pristotis</i> spp.	Marine
Pomacentridae	<i>Similiparma</i>	<i>Similiparma</i> spp.	Marine
Pomacentridae	<i>Stegastes</i>	<i>Stegastes</i> spp.	Marine
Pomacentridae	<i>Teixeirichthys</i>	<i>Teixeirichthys</i> spp.	Marine
Priacanthidae	<i>Cookeolus</i>	<i>Cookeolus</i> spp.	Marine
Priacanthidae	<i>Heteropriacanthu</i>	<i>Heteropriacanthus</i> spp.	Marine
Priacanthidae	<i>Priacanthus</i>	<i>Priacanthus</i> spp.	Marine
Priacanthidae	<i>Pristigenys</i>	<i>Pristigenys</i> spp.	Marine
Prochilodontidae	<i>Semaprochilodus</i>	<i>Semaprochilodus insignis</i>	Other Freshwater
Prochilodontidae	<i>Semaprochilodus</i>	<i>Semaprochilodus taeniurus</i>	Other Freshwater
Pseudochromidae	<i>Amsichthys</i>	<i>Amsichthys</i> spp.	Marine
Pseudochromidae	<i>Anisochromis</i>	<i>Anisochromis</i> spp.	Marine
Pseudochromidae	<i>Assiculoides</i>	<i>Assiculoides</i> spp.	Marine
Pseudochromidae	<i>Assiculus</i>	<i>Assiculus</i> spp.	Marine
Pseudochromidae	<i>Blennodesmus</i>	<i>Blennodesmus</i> spp.	Marine
Pseudochromidae	<i>Chlidichthys</i>	<i>Chlidichthys</i> spp.	Marine
Pseudochromidae	<i>Congrogadus</i>	<i>Congrogadus</i> spp.	Marine
Pseudochromidae	<i>Cypho</i>	<i>Cypho</i> spp.	Marine
Pseudochromidae	<i>Halidesmus</i>	<i>Halidesmus</i> spp.	Marine
Pseudochromidae	<i>Halimuraena</i>	<i>Halimuraena</i> spp.	Marine
Pseudochromidae	<i>Halimuraenoides</i>	<i>Halimuraenoides</i> spp.	Marine
Pseudochromidae	<i>Haliophis</i>	<i>Haliophis</i> spp.	Marine
Pseudochromidae	<i>Labracinus</i>	<i>Labracinus</i> spp.	Marine
Pseudochromidae	<i>Lubbockichthys</i>	<i>Lubbockichthys</i> spp.	Marine
Pseudochromidae	<i>Manonichthys</i>	<i>Manonichthys</i> spp.	Marine
Pseudochromidae	<i>Natalichthys</i>	<i>Natalichthys</i> spp.	Marine
Pseudochromidae	<i>Ogilbyina</i>	<i>Ogilbyina</i> spp.	Marine
Pseudochromidae	<i>Oxycercichthys</i>	<i>Oxycercichthys</i> spp.	Marine
Pseudochromidae	<i>Pectinochromis</i>	<i>Pectinochromis</i> spp.	Marine
Pseudochromidae	<i>Pholidochromis</i>	<i>Pholidochromis</i> spp.	Marine
Pseudochromidae	<i>Pictichromis</i>	<i>Pictichromis</i> spp.	Marine
Pseudochromidae	<i>Pseudochromis</i>	<i>Pseudochromis</i> spp.	Marine
Pseudochromidae	<i>Pseudoplesiops</i>	<i>Pseudoplesiops</i> spp.	Marine
Pseudochromidae	<i>Rusichthys</i>	<i>Rusichthys</i> spp.	Marine

Ptereleotridae		<i>Nemateleotris</i>	<i>Nemateleotris</i> spp.	Marine
Ptereleotridae		<i>Ptereleotris</i>	<i>Ptereleotris</i> spp.	Marine
Scaridae		<i>Bolbometopon</i>	<i>Bolbometopon</i> spp.	Marine
Scaridae		<i>Calotomus</i>	<i>Calotomus</i> spp.	Marine
Scaridae		<i>Cetoscarus</i>	<i>Cetoscarus</i> spp.	Marine
Scaridae		<i>Chlorurus</i>	<i>Chlorurus</i> spp.	Marine
Scaridae		<i>Cryptotomus</i>	<i>Cryptotomus</i> spp.	Marine
Scaridae		<i>Hipposcarus</i>	<i>Hipposcarus</i> spp.	Marine
Scaridae		<i>Leptoscarus</i>	<i>Leptoscarus</i> spp.	Marine
Scaridae		<i>Nicholsina</i>	<i>Nicholsina</i> spp.	Marine
Scaridae		<i>Scarus</i>	<i>Scarus</i> spp.	Marine
Scaridae		<i>Sparisoma</i>	<i>Sparisoma</i> spp.	Marine
Scorpaenidae	Pteroinae	<i>Dendrochirus</i>	<i>Dendrochirus</i> spp.	Marine
Scorpaenidae	Scorpaeninae	<i>Rhinopias</i>	<i>Rhinopias</i> spp.	Marine
Scorpaenidae		<i>Pterois</i>	<i>Pterois</i> spp.	Marine
Serranidae	Anthiinae	<i>Anthias</i>	<i>Anthias</i> spp.	Marine
Serranidae	Epinephalinae	<i>Cromileptes</i>	<i>Cromileptes</i> spp.	Marine
Siganidae		<i>Siganus</i>	<i>Siganus</i> spp.	Marine
Siluridae		<i>Kryptopterus</i>	<i>Kryptopterus bicirrhis</i>	Other Freshwater
Siluridae		<i>Kryptopterus</i>	<i>Kryptopterus macrocephalus</i>	Other Freshwater
Soleidae		<i>Brachirus</i>	<i>Brachirus</i> spp.	Marine
Strombidae		<i>Euprotomus</i>	<i>Monodactylus argenteus</i>	Other Freshwater
Strombidae		<i>Euprotomus</i>	<i>Monodactylus sebae</i>	Other Freshwater
Syngnathidae		<i>Acentronura</i>	<i>Acentronura</i> spp.	Marine
Syngnathidae		<i>Amphelikurus</i>	<i>Amphelikurus</i> spp.	Marine
Syngnathidae		<i>Anarchopterus</i>	<i>Anarchopterus</i> spp.	Marine
Syngnathidae		<i>Apterygocampus</i>	<i>Apterygocampus</i> spp.	Marine
Syngnathidae		<i>Bhanotia</i>	<i>Bhanotia</i> spp.	Marine
Syngnathidae		<i>Bryx</i>	<i>Bryx</i> spp.	Marine
Syngnathidae		<i>Bulbonaricus</i>	<i>Bulbonaricus</i> spp.	Marine
Syngnathidae		<i>Campichthys</i>	<i>Campichthys</i> spp.	Marine
Syngnathidae		<i>Choeroichthys</i>	<i>Choeroichthys</i> spp.	Marine
Syngnathidae		<i>Corythoichthys</i>	<i>Corythoichthys</i> spp.	Marine
Syngnathidae		<i>Cosmocampus</i>	<i>Cosmocampus</i> spp.	Marine
Syngnathidae		<i>Doryichthys</i>	<i>Doryichthys</i> spp.	Marine
Syngnathidae		<i>Doryrhamphus</i>	<i>Doryrhamphus</i> spp.	Marine
Syngnathidae		<i>Dunckerocampus</i>	<i>Dunckerocampus</i> spp.	Marine
Syngnathidae		<i>Enneacampus</i>	<i>Enneacampus</i> spp.	Marine
Syngnathidae		<i>Entelurus</i>	<i>Entelurus</i> spp.	Marine
Syngnathidae		<i>Festucalex</i>	<i>Festucalex</i> spp.	Marine
Syngnathidae		<i>Filicampus</i>	<i>Filicampus</i> spp.	Marine
Syngnathidae		<i>Halicampus</i>	<i>Halicampus</i> spp.	Marine
Syngnathidae		<i>Haliichthys</i>	<i>Haliichthys</i> spp.	Marine
Syngnathidae		<i>Heraldia</i>	<i>Heraldia</i> spp.	Marine
Syngnathidae		<i>Hippichthys</i>	<i>Hippichthys</i> spp.	Marine
Syngnathidae		<i>Histiogamphelus</i>	<i>Histiogamphelus</i> spp.	Marine

Syngnathidae	<i>Hypselognathus</i>	<i>Hypselognathus</i> spp.	Marine
Syngnathidae	<i>Ichthyocampus</i>	<i>Ichthyocampus</i> spp.	Marine
Syngnathidae	<i>Idiotropiscis</i>	<i>Idiotropiscis</i> spp.	Marine
Syngnathidae	<i>Kaupus</i>	<i>Kaupus</i> spp.	Marine
Syngnathidae	<i>Kimblaeus</i>	<i>Kimblaeus</i> spp.	Marine
Syngnathidae	<i>Kyonemichthys</i>	<i>Kyonemichthys</i> spp.	Marine
Syngnathidae	<i>Leptoichthys</i>	<i>Leptoichthys</i> spp.	Marine
Syngnathidae	<i>Leptonotus</i>	<i>Leptonotus</i> spp.	Marine
Syngnathidae	<i>Lissocampus</i>	<i>Lissocampus</i> spp.	Marine
Syngnathidae	<i>Maroubra</i>	<i>Maroubra</i> spp.	Marine
Syngnathidae	<i>Micrognathus</i>	<i>Micrognathus</i> spp.	Marine
Syngnathidae	<i>Microphis</i>	<i>Microphis</i> spp.	Marine
Syngnathidae	<i>Minyichthys</i>	<i>Minyichthys</i> spp.	Marine
Syngnathidae	<i>Mitotichthys</i>	<i>Mitotichthys</i> spp.	Marine
Syngnathidae	<i>Nannocampus</i>	<i>Nannocampus</i> spp.	Marine
Syngnathidae	<i>Nerophis</i>	<i>Nerophis</i> spp.	Marine
Syngnathidae	<i>Notiocampus</i>	<i>Notiocampus</i> spp.	Marine
Syngnathidae	<i>Penetopteryx</i>	<i>Penetopteryx</i> spp.	Marine
Syngnathidae	<i>Phoxocampus</i>	<i>Phoxocampus</i> spp.	Marine
Syngnathidae	<i>Phycodurus</i>	<i>Phycodurus</i> spp.	Marine
Syngnathidae	<i>Phyllopteryx</i>	<i>Phyllopteryx</i> spp.	Marine
Syngnathidae	<i>Pseudophallus</i>	<i>Pseudophallus</i> spp.	Marine
Syngnathidae	<i>Pugnaso</i>	<i>Pugnaso</i> spp.	Marine
Syngnathidae	<i>Siokunichthys</i>	<i>Siokunichthys</i> spp.	Marine
Syngnathidae	<i>Solegnathus</i>	<i>Solegnathus</i> spp.	Marine
Syngnathidae	<i>Stigmatopora</i>	<i>Stigmatopora</i> spp.	Marine
Syngnathidae	<i>Stipecampus</i>	<i>Stipecampus</i> spp.	Marine
Syngnathidae	<i>Syngnathoides</i>	<i>Syngnathoides</i> spp.	Marine
Syngnathidae	<i>Syngnathus</i>	<i>Syngnathus</i> spp.	Marine
Syngnathidae	<i>Trachyrhamphus</i>	<i>Trachyrhamphus</i> spp.	Marine
Syngnathidae	<i>Urocampus</i>	<i>Urocampus</i> spp.	Marine
Syngnathidae	<i>Vanacampus</i>	<i>Vanacampus</i> spp.	Marine
Telmatherinidae	<i>Marosatherina</i>	<i>Marosatherina ladigesi</i>	Other Freshwater
Tetraodontidae	<i>Canthigaster</i>	<i>Canthigaster</i> spp.	Marine
Toxotidae	<i>Toxotes</i>	<i>Toxotes jaculatrix</i>	Other Freshwater
Zanclidae	<i>Zanclus</i>	<i>Zanclus cornutus</i>	Marine
Zenarchopteridae	<i>Dermogenys</i>	<i>Dermogenys pusilla</i>	Other Freshwater

Appendix D

Laboratory Test Data from Phase 1

Dwarf Gourami ¹	Receive date and sample no.	Species	Specimen type	Test type	Test result
1 (2e)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Formalin fixed tissue	General Histology	No significant lesions
2 (2f)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Formalin fixed tissue	General Histology	No significant lesions
3 (2g)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Formalin fixed tissue	General Histology	Granulomas in kidney. Limited range of tissues
4 (2h)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Formalin fixed tissue	General Histology	Limited range of tissues. No significant lesions
5 (2d)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Ethanol fixed tissue	Megalocytivirus – nested PCR (Rimmer)	Positive
5 (2d)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Positive
6 (2a)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
7 (2b)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
8 (2c)	13-02061 30-Jul-2013	<i>Trichogaster lalius</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
9 (2d)	13-01881 11-Jul-2013	<i>Trichogaster lalius</i>	Formalin fixed tissue	General Histology	A number of granulomas scattered throughout some unidentifiable tissue (gut wall?)
10 (2e)	13-01881 11-Jul-2013	<i>Trichogaster lalius</i>	Formalin fixed tissue	General Histology	No obvious lesions
11 (2f)	13-01881 11-Jul-2013	<i>Trichogaster lalius</i>	Formalin fixed tissue	General Histology	Limited range of poorly fixed tissues. No obvious lesions. Little fish such as these should be fixed properly and submitted whole
12 (2a)	13-01881 11-Jul-2013	<i>Trichogaster lalius</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
13 (2b)	13-01881 11-Jul-2013	<i>Trichogaster lalius</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
14 (2c)	13-01881 11-Jul-2013	<i>Trichogaster lalius</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative

Platy ²	Receive date and sample no.	Species	Specimen type	Test type	Test result
1 (2e)	13-02060 30-Jul-2013	<i>Xiphophorus maculatus</i>	Formalin fixed tissue	General Histology	No significant lesions
2 (2f)	13-02060 30-Jul-2013	<i>Xiphophorus maculatus</i>	Formalin fixed tissue	General Histology	No significant lesions
3 (2g)	13-02060 30-Jul-2013	<i>Xiphophorus maculatus</i>	Formalin fixed tissue	General Histology	No significant lesions
4 (2h)	13-02060 30-Jul-2013	<i>Xiphophorus maculatus</i>	Formalin fixed tissue	General Histology	No significant lesions
5 (2b)	13-02060 30-Jul-2013	<i>Xiphophorus maculatus</i>	Ethanol fixed tissue	Megalocytivirus – nested PCR (Rimmer)	Positive
(2b)	13-02060 30-Jul-2013	<i>Xiphophorus maculatus</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Positive
6 (2a)	13-02060 30-Jul-2013	<i>Xiphophorus maculatus</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
7 (2c)	13-02060 30-Jul-2013	<i>Xiphophorus maculatus</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
8 (2d)	13-02060	<i>Xiphophorus</i>	Ethanol	Megalocytivirus –	Negative

¹ Dwarf Gouramis were visually healthy fish purchased for the purposes of trial 1.

² Platys were visually healthy fish purchased for the purposes of trial 1.

	30-Jul-2013	<i>maculatus</i>	fixed tissue	TaqMan PCR	
9 (3e)	13-01877 11-Jul-2013	<i>Xiphophorus maculatus</i>	Fixed tissue	General Histology	No significant lesions. Specimen very poorly fixed. Female with live young in body cavity
10 (3f)	13-01877 11-Jul-2013	<i>Xiphophorus maculatus</i>	Fixed tissue	General Histology	No significant lesions. Specimen very poorly fixed. Possibly male
11 (3g)	13-01877 11-Jul-2013	<i>Xiphophorus maculatus</i>	Fixed tissue	General Histology	Male. Poorly fixed. Large no. of parasites encysted in a fibrous capsule in liver, and in ventral body cavity. Either nematodes or acanthocephalans; poor fixation
12 (3h)	13-01877 11-Jul-2013	<i>Xiphophorus maculatus</i>	Fixed tissue	General Histology	Probably female. Poor fixation. No obvious lesions
13 (3a)	13-01877 11-Jul-2013	<i>Xiphophorus maculatus</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
14 (3b)	13-01877 11-Jul-2013	<i>Xiphophorus maculatus</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
15 (3c)	13-01877 11-Jul-2013	<i>Xiphophorus maculatus</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
16 (3d)	13-01877 11-Jul-2013	<i>Xiphophorus maculatus</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative

Goldfish ³	Receive date and sample no.	Species	Specimen type	Test type	Test result
1 (1a)	13-01888 26-Jun-2013	<i>Carassius auratus</i>	Fixed tissue	General Histology	No lesions in liver, spleen, kidney or pancreas. Little functional tissue in gills; lamellae obliterated. Hydropic degeneration of the lamellar epithelium, but all changes may be compounded by poor fixation technique
2 (1b)	13-01888 26-Jun-2013	<i>Carassius auratus</i>	Fixed tissue	General Histology	Gills poorly fixed; hyperplasia of epithelium of gill-rakers, and moderate inflammatory lesion in underlying submucosa. No other lesions observed
3 (1c)	13-01888 26-Jun-2013	<i>Carassius auratus</i>	Fixed tissue	General Histology	Occasional granulomas in the renal, splenic and hepatic interstitium. Gills similar to 1a
4 (1d)	13-01888 26-Jun-2013	<i>Carassius auratus</i>	Fixed tissue	General Histology	Large parasite in kidney; haemorrhage in parenchyma. Parasite full of ova. Gills similar to 1a and 1c
5 (1e)	13-01888 26-Jun-2013	<i>Carassius auratus</i>	Fixed tissue	General Histology	Single granuloma in the splenic parenchyma. Gills similar to 1a and 1c
6 (1f)	13-01888 26-Jun-2013	<i>Carassius auratus</i>	Fixed tissue	General Histology	Single granuloma in the renal parenchyma. Gills similar to 1a and 1c
Fish 1	13-01873 10-Jul-2013	<i>Carassius auratus</i>	Whole fish	General Bacteriology isolation and I.D.	No <i>Aeromonas salmonicida</i> detected
Fish 1	13-01873 10-Jul-2013	<i>Carassius auratus</i>	Whole fish	<i>Aeromonas salmonicida</i> – PCR (AP)	Negative
Fish 1	13-01873 10-Jul-2013	<i>Carassius auratus</i>	Whole fish	<i>Aeromonas salmonicida</i> – PCR (PAAS)PCR (PAAS)	Negative
Fish 1	13-01873 10-Jul-2013	<i>Carassius auratus</i>	Whole fish	<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> – PCR (Miyata)	Negative
Fish 2	13-01873 10-Jul-2013	<i>Carassius auratus</i>	Whole fish	General Bacteriology isolation and I.D.	No <i>Aeromonas salmonicida</i> detected
Fish 2	13-01873 10-Jul-2013	<i>Carassius auratus</i>	Whole fish	<i>Aeromonas salmonicida</i> – PCR (AP)	Negative
Fish 2	13-01873	<i>Carassius</i>	Whole	<i>Aeromonas salmonicida</i>	Negative

³ Goldfish were visually healthy fish purchased for the purposes of trial 1.

	10-Jul-2013	<i>auratus</i>	<i>fish</i>	– PCR (PAAS)	
Fish 2	13-01873 10-Jul-2013	<i>Carassius auratus</i>	<i>Whole fish</i>	<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> – PCR (Miyata)	Negative
Fish 3	13-01873 10-Jul-2013	<i>Carassius auratus</i>	<i>Whole fish</i>	<i>General Bacteriology isolation and I.D.</i>	<i>No Aeromonas salmonicida detected</i>
Fish 4	13-01873 10-Jul-2013	<i>Carassius auratus</i>	<i>Whole fish</i>	<i>General Bacteriology isolation and I.D.</i>	<i>No Aeromonas salmonicida detected</i>
Fish 5	13-01873 10-Jul-2013	<i>Carassius auratus</i>	<i>Whole fish</i>	<i>General Bacteriology isolation and I.D.</i>	<i>No Aeromonas salmonicida detected</i>
Fish 6	13-01873 10-Jul-2013	<i>Carassius auratus</i>	<i>Whole fish</i>	<i>General Bacteriology isolation and I.D.</i>	<i>No Aeromonas salmonicida detected</i>
13 (Fish Bag A)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	General Bacteriology isolation and I.D.	No <i>Aeromonas salmonicida</i> isolated
14 (Fish Bag B)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	General Bacteriology isolation and I.D.	No <i>Aeromonas salmonicida</i> isolated
15 (Fish Bag C)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	General Bacteriology isolation and I.D.	No <i>Aeromonas salmonicida</i> isolated
16 (Fish Bag D)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	General Bacteriology isolation and I.D.	No <i>Aeromonas salmonicida</i> isolated
17 (Fish Bag E)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	General Bacteriology isolation and I.D.	No <i>Aeromonas salmonicida</i> isolated
18 (Fish Bag F)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	General Bacteriology isolation and I.D.	No <i>Aeromonas salmonicida</i> isolated
19 (Fish A)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	Virus isolation by cell culture – EPC cell line	Negative for SVCV No cytopathic effect observed
20 (Fish B)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	Virus isolation by cell culture – EPC cell line	SVCV negative No cytopathic effect observed
21 (Fish C)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	Virus isolation by cell culture – EPC cell line	SVCV negative No cytopathic effect observed
22 (Fish D)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	Virus isolation by cell culture – EPC cell line	SVCV negative No cytopathic effect observed
(Fish D)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	<i>Aeromonas salmonicida</i> – PCR (AP)	Negative
(Fish D)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	<i>Aeromonas salmonicida</i> – PCR (PAAS)	Negative
(Fish D)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> – PCR (Miyata)	Negative
23 (Fish E)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	Virus isolation by cell culture – EPC cell line	SVCV negative No cytopathic effect observed
24 (Fish F)	13-01738 26-Jun-2013	<i>Carassius auratus</i>	Fresh Tissue	Virus isolation by cell culture – EPC cell line	SVCV negative No cytopathic effect observed
1	13-01889 11/7/13	<i>Carassius auratus</i>	Tissue	SVCV - Virus isolation by cell culture – EPC cell line	No cytopathic effect observed
2	13-01889 11/7/13	<i>Carassius auratus</i>	Tissue	SVCV - Virus isolation by cell culture – EPC cell line	No cytopathic effect observed
3	13-01889 11/7/13	<i>Carassius auratus</i>	Tissue	SVCV - Virus isolation by cell culture – EPC cell line	No cytopathic effect observed
4	13-01889 11/7/13	<i>Carassius auratus</i>	Tissue	SVCV - Virus isolation by cell culture – EPC cell line	No cytopathic effect observed
5	13-01889 11/7/13	<i>Carassius auratus</i>	Tissue	SVCV - Virus isolation by cell culture – EPC cell line	No cytopathic effect observed
6	13-01889 11/7/13	<i>Carassius auratus</i>	Tissue	SVCV - Virus isolation by cell culture – EPC cell line	No cytopathic effect observed

1 Gold 2a	13-02058 30/7/13	<i>Carassius auratus</i>	Formal in fixed tissue	Histo	No obvious lesions. Lamellae of gills very cellular but poor fixation makes interpretation difficult
2 Gold 2b	13-02058 30/7/13	<i>Carassius auratus</i>	Formal in fixed tissue	Histo	Spleen and pancreas; no significant lesions. Gills as same as above
3 Gold 2c	13-02058 30/7/13	<i>Carassius auratus</i>	Formal in fixed tissue	Histo	Pancreas; no significant lesions. Multifocal granulomas, each with necrotic centre, scattered throughout spleen. In kidney, acute foci of necrosis and pigmentation. Gills as same as Gold 2a. Some bacteria present in the splenic granulomas. Ziehl-Nielson stain did not reveal any acid-fast bacilli in the granulomas
4 Gold 2d	13-02058 30/7/13	<i>Carassius auratus</i>	Formal in fixed tissue	Histo	Liver, pancreas, spleen: no significant lesions. Multiple discrete granulomas throughout the kidney. Gills poorly fixed, but no obvious significant lesions
5 Gold 2e	13-02058 30/7/13	<i>Carassius auratus</i>	Formal in fixed tissue	Histo	Multifocal granulomas, each with a necrotic centre, throughout the kidney, and also the spleen. Gills same as Gold 2d
6 Gold 2f	13-02058 30/7/13	<i>Carassius auratus</i>	Formal in fixed tissue	Histo	Liver, pancreas: no significant lesions. Multifocal granulomas, each with a necrotic centre, throughout the kidney (few in spleen). Gills poorly fixed, no obvious significant lesions

The following species are seized samples due to non-compliance issues.

Saddle butterflyfish	Receive date and sample no.	Species	Specimen type	Test type	Test result
1 (191971a)	13-01739 26-Jun-2013	<i>Chaetodon ephippium</i>	Formalin fixed tissue	General Histology	Selected tissues. No visible lesions in gills or spleen. No other tissues submitted
2 (191971b)	13-01739 26-Jun-2013	<i>Chaetodon ephippium</i>	Formalin fixed tissue		No visible lesions in kidney, spleen. In gills, mild chronic inflammation of the submucosa of the gill-rakers, and a single encysted parasite is present at the proximal end of one branchial filament. No other tissues submitted

Blind cave tetra	Receive date and sample no.	Species	Specimen type	Test type	Test result
1 (191970a)	13-01740 26-Jun-2013	<i>Astyanax jordani</i>	Formalin fixed tissue	General Histology	Mid-sagittal section of fish. No significant lesions in brain, heart, liver, kidney, pancreas, gut or gills
2 (191970b)	13-01740 26-Jun-2013	<i>Astyanax jordani</i>	Formalin fixed tissue	General Histology	Mid-sagittal section of fish. Mild chronic focal pancreatitis and mild chronic branchitis. No other visible lesions

Lyretail Anthias	Receive date and sample no.	Species	Specimen type	Test type	Test result
1 (204032a)	13-01741 26-Jun-2013	<i>Pseudanthias cheirospilos</i>	Formalin fixed tissue	General Histology	Focal area of vacuolation in the brain-stem, ventral to the optic lobe. In absence of negative control fish hard to know if this is normal or not. Few loci of non-supportive

					pancreatitis. No other visible lesions
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Sailfin molly	Receive date and sample no.	Species	Specimen type	Test type	Test result
1 (216644c)	13-01742 26-Jun-2013	<i>Poecilia latipinna</i>	Formalin fixed tissue	General Histology	No visible lesions
2 (216644d)	13-01742 26-Jun-2013	<i>Poecilia latipinna</i>	Formalin fixed tissue	General Histology	Must be live-bearing fish as there are about 6 immature fish, each with own yolk sac, in abdomen. No visible lesions
3 (216644a)	13-01742 26-Jun-2013	<i>Poecilia latipinna</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative
4 (216644b)	13-01742 26-Jun-2013	<i>Poecilia latipinna</i>	Ethanol fixed tissue	Megalocytivirus – TaqMan PCR	Negative