ACERA II 1206G Ornamental Finfish Import Reform Program
A sampling framework and trial for the surveillance program
Final Report (OID1)

1.   Introduction

The rules of international trade mandate that biosecurity risk management policies be supported by valid scientific data and risk analysis except where the international standard is exceeded (WTO, 2010).

The current risk management measures for the importation of ornamental fish are based on the Import Risk Analysis (IRA) on live ornamental finfish (Kahn et al., 1999) and the current requirements for importation of ornamental fish to Australia include pre-border health certification by the competent authority of the exporting country and a mandatory on-arrival quarantine period of one to three weeks (depending on the species) in privately owned and registered Class 7.1 quarantine-approved premises (QAPs). Quarantined fish are observed for signs of disease, but are not directly tested during this time.

The Department of Agriculture, Fisheries and Forestry (DAFF) Animal Biosecurity and Animal Import Operations branches completed an inhouse review of current post-arrival import conditions for imported ornamental fish and concluded that the current post-arrival quarantine arrangements, based on an isolation period at QAPs, are not effective. Specifically, the review found that the current system can not address risks associated with subclinically infected fish. The shortcomings of the current arrangements are highlighted by significant disease incursions in recent times, including the establishment of goldfish herpes virus and the periodic detection of gourami iridovirus in imported fish post-quarantine in Australia.

In recognition of this problem and in an attempt to better manage disease risks, DAFF is proposing changes to the way it manages the disease risks associated with imported ornamental fish. The proposed changes include the introduction of on-arrival health surveillance that will allow DAFF to monitor 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 specific diseases and pathogens, the proposed surveillance program will make more use of epidemiological evidence to identify pests and disease of potential biosecurity concern (i.e., new and emerging pests). The establishment of an on-arrival health surveillance program will allow DAFF to address the systems limitations identified in its inhouse review, as well as addressing the findings of the provisional final IRA on *Importation of Freshwater Ornamental Fish: Review of Biosecurity Risks Associated with Gourami Iridovirus and Related Viruses* (Biosecurity Australia, 2010) and the findings of the Interim Inspector General of Biosecurity (IIGB) report.

2.   Sampling Framework

The proposed surveillance program will monitor the exporting country's compliance with the import conditions to provide fish with no clinical signs of significant pests or diseases (as attested to in the health certification that accompanies each consignment). General health will be assessed using histopathological techniques, and a health index will be developed. These data will support monitoring for emerging diseases and pathogens. The surveillance program will be flexible to allow the inclusion of targeted testing for new pathogens of biosecurity concern. The inclusion of new pathogens of biosecurity concern for ongoing, targeted surveillance will be on the basis of a risk assessment that shows the pathogen represents an unacceptable risk to Australia.

The mechanisms of the proposed surveillance program include:

- all shipments of freshwater and marine ornamental fish will be inspected on arrival (as is the current practice);
- bags of fish in consignments showing significant signs of disease will be sampled during on-arrival inspection and the remaining fish in the consignment will be destroyed or exported;

- bags of fish in all other consignments will be subject to random sampling during on-arrival inspection before the remaining fish are released from quarantine;
- fish samples will be sent to participating laboratories for testing for specific pathogens of biosecurity concern and histopathology as needed;
- the information obtained from the samples will be analysed and appropriate action taken to manage any biosecurity risk at the source; and
- sampling rates will be adjusted for particular fish risk groups, exporter locations, et cetera in order to gather additional information/evidence on changed risks as deemed necessary.

Current risk management measures for the importation of ornamental fish are based on the IRA on live ornamental finfish (Kahn et al., 1999). DAFF's gourami iridovirus IRA (Biosecurity Australia, 2010) reviewed the policy on importation of freshwater ornamental fish with respect to iridoviruses as a result of new scientific information regarding susceptible species and carrier status. The grouping of fish taxa that resulted from the IRAs was used as the basis for identifying fish 'risk groups' for the surveillance program. The risk groups are:

- Goldfish
- Gouramis
- Cichlids
- Poecilids
- Other Freshwater species (meaning all freshwater species not listed above)
- Marine species

The current practice of categorising consignments and bags of fish on arrival as 'compliant' or 'non-compliant' will provide the basis for distinguishing two sample testing 'streams', which are summarised in Table 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 (see Section 3). The sampling framework for the surveillance program is to target only non-compliances due to signs of disease for Stream 2 sampling.

Table 1. The two sample testing streams of the surveillance program

	Stream 1 (compliant)	**Stream 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	Compliant consignments	Non-compliant consignments (due to signs of disease)
Trigger	To be established. Data captured in the trial will be analysed and used to inform this decision	To be established. Data captured in the trial will be analysed and used to inform this decision
Testing	PCR and bacteriological testing for pathogens of biosecurity concern, which are currently: *A. salmonicida*, SVCV and Megalocytivirus	Histopathology and bacteriology for general health and, depending on these results, potentially 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)
Sampling approach	Random sampling	Targeted sampling, i.e., biased towards the unhealthiest fish

DAFF will undertake a trial of the sampling framework for the surveillance program. The trial will test the operational feasibility of sample collection and laboratory testing, and will collect information to inform the statistical design of the surveillance program. The trial will be undertaken in two phases to progressively test elements of the program and to expand the trial to multiple locations across Australia. Each phase will be planned to yield information to inform the design of the next phase and, ultimately, to determine the design of the surveillance program for post-trial implementation.

Both sampling streams will be tested during the trial. Stream 2 will be the subject of phase 1 of the trial and Stream 1 will be included during the second phase once volunteer importers are available. Stream 2 is intentionally biased (targeted) in an attempt to determine which diseases, if any, are the cause of observed morbidity in non-compliant bags of fish. In contrast, Stream 1 is a random sample to estimate the prevalence of pathogens of biosecurity concern by source country and risk group.

The trial will be somewhat different in structure and content to the post-trial surveillance program. This is because various *potential* elements of the surveillance program will be trialled, assessed and compared. The results of this process will inform the development of the surveillance program, but the final form of the program will be a product of what is learnt during the trial and is not yet known.

The first phase of the trial is the subject of the ACERA 1206G project and the remainder of this document; the second phase (and any subsequent phases of the trial) forms the basis of a complimentary Centre of Excellence for Biosecurity Risk Analysis (CEBRA) proposal, which is an extension of the current project.

3. Trial Phase 1

Sample population

Phase 1 of the trial will be restricted to Stream 2 sampling. Its main purpose is 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 is not critical for this project. Early on in the project, though, we did have some preliminary discussions with Graham Hepworth at the Statistical Consulting Centre (University of Melbourne). These discussions will become more pertinent when the second phase (and subsequent phases) is being designed because random sampling will be integrated from this point.

To test the operational characteristics of the sampling framework in this first phase of the trial, we will target only those bags of fish that would otherwise be destroyed due to non-compliance with current Australian import requirements. Broadly, non-compliance refers to a failure of the fish inspection or the documentation verification (or both) 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:
    - Significant mortalities in a bag (>10%)
    - Loss of colouring or darkening
    - Abnormal swimming behaviours, hovering at the surface
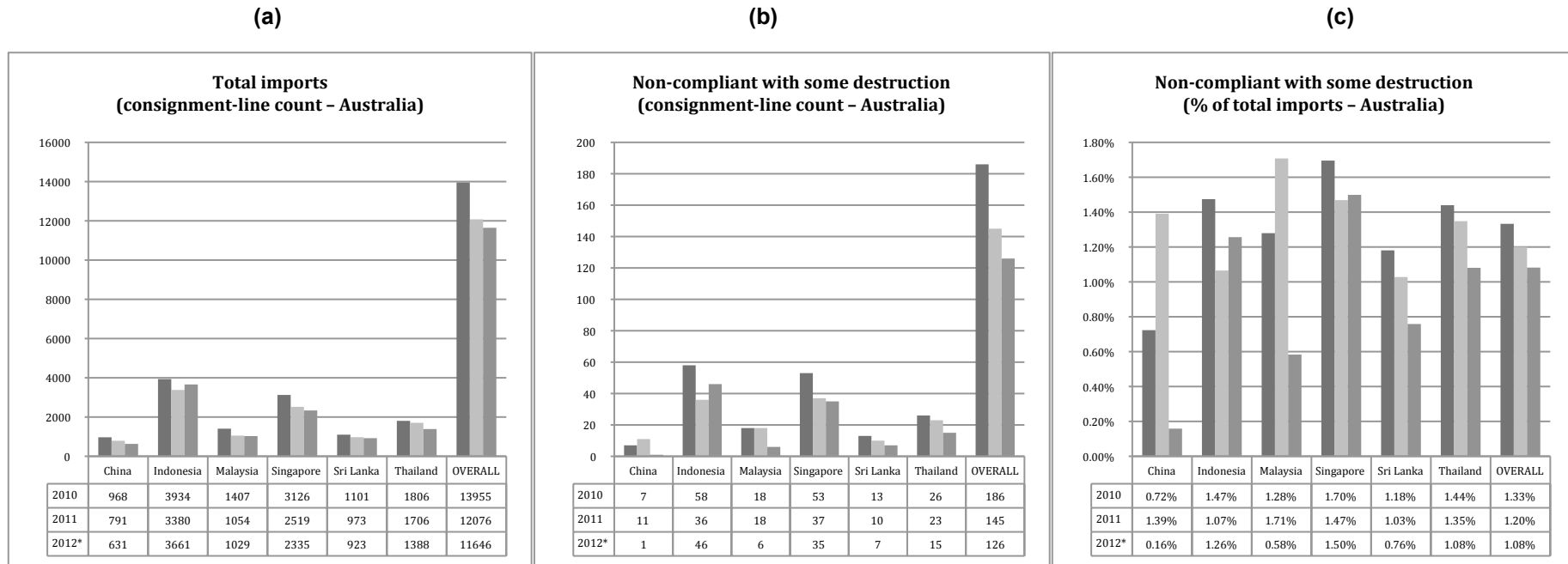   - Lethargy or hyper/hypo-activity

- o Fin burn/congestion/erosion
- o Abnormal respiratory activity such as rapid gulping
- o Loss of balance, swimming on the side
- o Excess mucous secretion
- o Abnormal swelling
- o Bulging eyes
- o Reddening/hemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes
- o Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss

To estimate the number of non-compliant bags of fish that might be available due to non-compliance, we have reviewed data from the AQIS Import Management System (AIMS). We looked only at the six ornamental fish exporting countries of greatest interest to DAFF: China, Indonesia, Malaysia, Singapore, Sri Lanka and Thailand. We have presented aggregated results for all imports to Australia, as well as specific results for Victoria (i.e., Melbourne), which is the phase 1 trial site because of its relatively high level of activity.
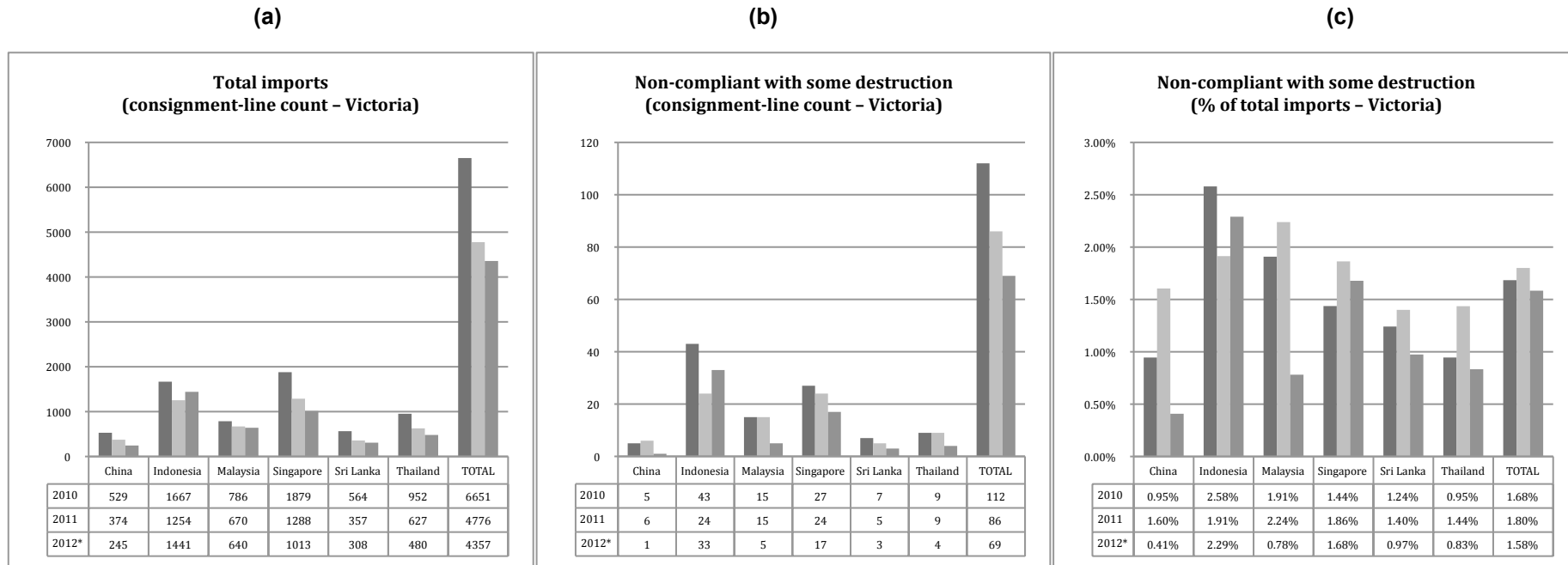
The data (Figures 1 and 2) show that the total number of imported consignments has decreased in the past three years, as has the number of non-compliant consignments at both the Australian and Victorian levels. Overall, this has resulted in a modest declining trend in the rate (%) of non-compliances at the Australian level, but a less clear trend in Victoria. The overall non-compliance rate in Victoria is higher than the Australian average for all years in the presented data. This may be due to the very low rates of non-compliance in NSW (data not presented here). The variation in the size of the non-compliant population by importing country can be observed in Figures 1 and 2. Figure 2b shows the number of non-compliance destruction events that have occurred over the past three years, which provides an indication of the likely available sample rate for Stream 2.

The available sample rate of approximately one non-compliance per week is lower than would be ideal for phase 1 of the trial. As such, *all* non-compliant bags (i.e., for any reason listed above) that is directed for destruction by the importer will be sampled during phase 1 of the trial.

Figure 1. The total number of consignments entering Australia (a), the total number of non-compliant consignments that were destroyed or partially destroyed in Australia (b), the total percentage of non-compliant consignments that were destroyed or partially destroyed in Australia (c) for the six importing countries of interest from 2010 to 2012 (linear extrapolation for Sep to Dec 2012 as these date were unavailable).

(a)   **(b)**   **(c)**

Total imports (consignment-line count – Australia)

	China	Indonesia	Malaysia	Singapore	Sri Lanka	Thailand	OVERALL
2010	968	3934	1407	3126	1101	1806	13955
2011	791	3380	1054	2519	973	1706	12076
2012*	631	3661	1029	2335	923	1388	11646

Non-compliant with some destruction (consignment-line count – Australia)

	China	Indonesia	Malaysia	Singapore	Sri Lanka	Thailand	OVERALL
2010	7	58	18	53	13	26	186
2011	11	36	18	37	10	23	145
2012*	1	46	6	35	7	15	126

Non-compliant with some destruction (% of total imports – Australia)

	China	Indonesia	Malaysia	Singapore	Sri Lanka	Thailand	OVERALL
2010	0.72%	1.47%	1.28%	1.70%	1.18%	1.44%	1.33%
2011	1.39%	1.07%	1.71%	1.47%	1.03%	1.35%	1.20%
2012*	0.16%	1.26%	0.58%	1.50%	0.76%	1.08%	1.08%

Figure 2. The total number of consignments entering Victoria (a), the total number of non-compliant consignments that were destroyed or partially destroyed in Victoria (b), the total percentage of non-compliant consignments that were destroyed or partially destroyed in Victoria (c) for the six importing countries of interest from 2010 to 2012 (linear extrapolation for Sep to Dec 2012 as these date were unavailable).

(a)

Total imports
(consignment-line count – Victoria)

	China	Indonesia	Malaysia	Singapore	Sri Lanka	Thailand	TOTAL
2010	529	1667	786	1879	564	952	6651
2011	374	1254	670	1288	357	627	4776
2012*	245	1441	640	1013	308	480	4357

(b)

Non-compliant with some destruction
(consignment-line count – Victoria)

	China	Indonesia	Malaysia	Singapore	Sri Lanka	Thailand	TOTAL
2010	5	43	15	27	7	9	112
2011	6	24	15	24	5	9	86
2012*	1	33	5	17	3	4	69

(c)

Non-compliant with some destruction
(% of total imports – Victoria)

	China	Indonesia	Malaysia	Singapore	Sri Lanka	Thailand	TOTAL
2010	0.95%	2.58%	1.91%	1.44%	1.24%	0.95%	1.68%
2011	1.60%	1.91%	2.24%	1.86%	1.40%	1.44%	1.80%
2012*	0.41%	2.29%	0.78%	1.68%	0.97%	0.83%	1.58%

Fish health data

While trialling practical implementation is the main purpose of Phase 1 of the trial, data capture relating to fish health status is also important, as it will help shape decision-making beyond this phase. Upon inspection of bags of fish at the border in Melbourne, DAFF inspectors will record their visual observations of fish health that can be used to estimate ill health and disease prevalence across the entire imported ornamental fish population. Morbidity and mortality rates will be estimated at the bag level (see Datasheet A), signs of ill health will be recorded at the bag level (see Datasheet B) and signs of ill health will be recorded for each sampled fish (see Datasheet C). Recording to these datasheets will be by exception, so if there are no morbid or dead fish in an entire consignment then no sheets will be completed. Packing lists showing the number of fish of each species and the number of bags will be collected for every consignment, even those that show no morbidity or mortality.

In addition to estimating rates of morbidity and mortality, these data could potentially highlight the signs of ill health that are indicative of clinical disease by aligning the results of laboratory testing with the visual data. This is particularly important when the surveillance program is eventually implemented because decision-making will occur at the border on the basis of visual observations only (QAPs will no longer exist under the proposed surveillance model).

The data described above will be collected in hardcopy form during phase 1 of the trial. A research assistant will digitise the datasheets. The results will be stored on existing AIMS and incidents databases, and on either the ornamental fish import reform network (OFIN) on Sharepoint or Excel spreadsheets on local drives during Phase 1 of the trial.

Sample collection, preparation and testing

After the fish health data have been recorded and DAFF inspectors have detected a non-compliance that is directed for destruction by the importer, sampling of fish from the non-compliant bag will be triggered. The steps that will be taken to collect, prepare, transport and test the fish samples (and to record the associated data) are presented as a flow diagram in Figure 3. Detailed Work Instructions have been prepared for the critical points (such as sampling and laboratory protocols) to provide greater detail around these activities. The planned sampling and testing structure for Phase 1 of the trial is presented in Figure 4.

Implementation

Phase 1 of the trial commenced in Melbourne (south east region) on 6 May 2013 for 8 weeks. Upon implementation, the operation of the program will be closely monitored by both DAFF and ACERA. The end of this phase of the trial marks the end of this project (and ACERA, which transitions to CEBRA on 1 July, 2013).

Visual observation and laboratory data gathered will be analysed and, together with information learned about sample effort, cost and accessibility, they will help shape the framework for Phase 2 of the trial and beyond. Full details about the analysis of Phase 1 of the trial, the mechanisms of Phase 2 of the trial, and post-trial design and implementation forms the basis of a CEBRA project, which is an extension of the current project.

Literature Cited

Biosecurity Australia (2010). Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses – Provisional final import risk analysis report. Biosecurity Australia, Canberra, Australia.

Go, J., Lancaster, M., Deece, K., Dhungyel, O., Whittington, R. (2006). The molecular epidemiology of iridovirus in Murray cod (*Maccullochella peelii peelii*) and dwarf gourami (*Colisa lalia*) from distant biogeographical regions suggests a link between trade in ornamental fish and emerging iridoviral diseases. Molecular and Cellular Probes 20: 212–222.

Kahn, S. A., Wilson, D. W., Perera, R. P., Hayder, H., Gerrity, S. E. (1999). Import risk analysis on live ornamental finfish. Australian Quarantine and Inspection Service, Canberra, Australia.

Whittington, R. J., Chong, R. (2007). Global trade in ornamental fish from an Australian perspective: The case for revised import risk analysis and management strategies. Preventive Veterinary Medicine 81: 92–116.

WTO (2010). The WTO Agreements Series Sanitary and Phytosanitary Measures. World Trade Organization, Geneva, Switzerland.

Ornamental Fish – Data Sheet A
To be completed for all bags of fish that have any morbidity or mortality, including bags deemed to be a major non-compliance

Quarantine Entry (AIMS) Code...

	Row ID	Species (Scientific Name)	Number of Fish in Bag	Number of Morbid Fish	Number of Dead Fish	Signs of Ill Health? [Blank = No]		Non-compliant/Destruction? [Blank = No]		Notes (about the bag)
Each row relates to one bag of fish	1						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	2						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	3						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	4						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	5						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	6						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	7						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	8						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	9						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	10						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	11						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	12						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	13						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	14						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	
	15						*If Yes –> Sheet B*		*If Yes –> Sheet C & PDI*	

General Notes...

Ornamental Fish – Data Sheet B

To be completed for all bags that contain any fish with any signs of ill health, including bags deemed to be a major non-compliance

Quarantine Entry (AIMS) Code..

Each column below relates to one bag of fish

Row ID (from Sheet A)..........	**Row ID (from Sheet A)**..........	**Row ID (from Sheet A)**..........
Sign(s) of Ill Health in the Bag? [Tick all that apply]	**Sign(s) of Ill Health in the Bag?** [Tick all that apply]	**Sign(s) of Ill Health in the Bag?** [Tick all that apply]
☐ Significant mortalities in the bag	☐ Significant mortalities in the bag	☐ Significant mortalities in the bag
☐ Loss of colouring or darkening	☐ Loss of colouring or darkening	☐ Loss of colouring or darkening
☐ Abnormal swimming behaviours, hovering at the surface	☐ Abnormal swimming behaviours, hovering at the surface	☐ Abnormal swimming behaviours, hovering at the surface
☐ Lethargy or hyper/hypo-activity	☐ Lethargy or hyper/hypo-activity	☐ Lethargy or hyper/hypo-activity
☐ Fin burn/congestion/erosion	☐ Fin burn/congestion/erosion	☐ Fin burn/congestion/erosion
☐ Respiratory activity such as rapid gulping	☐ Respiratory activity such as rapid gulping	☐ Respiratory activity such as rapid gulping
☐ Loss of balance, swimming on the side	☐ Loss of balance, swimming on the side	☐ Loss of balance, swimming on the side
☐ Excess mucous secretion	☐ Excess mucous secretion	☐ Excess mucous secretion
☐ Abnormal swelling	☐ Abnormal swelling	☐ Abnormal swelling
☐ Bulging eyes	☐ Bulging eyes	☐ Bulging eyes
☐ Reddening/hemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes)	☐ Reddening/hemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes)	☐ Reddening/hemorrhaging (red spots or areas, especially around the mouth, anus, fin margins and eyes)
☐ Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss	☐ Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss	☐ Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss
Do you think ill health was caused by transport stress?	**Do you think ill health was caused by transport stress?**	**Do you think ill health was caused by transport stress?**
No Yes	No Yes	No Yes

General Notes...

Ornamental Fish – Data Sheet C

To be completed for each non-compliant bag that has been nominated for destruction, for each fish sampled from the bag (A up to J, depending how many fish)
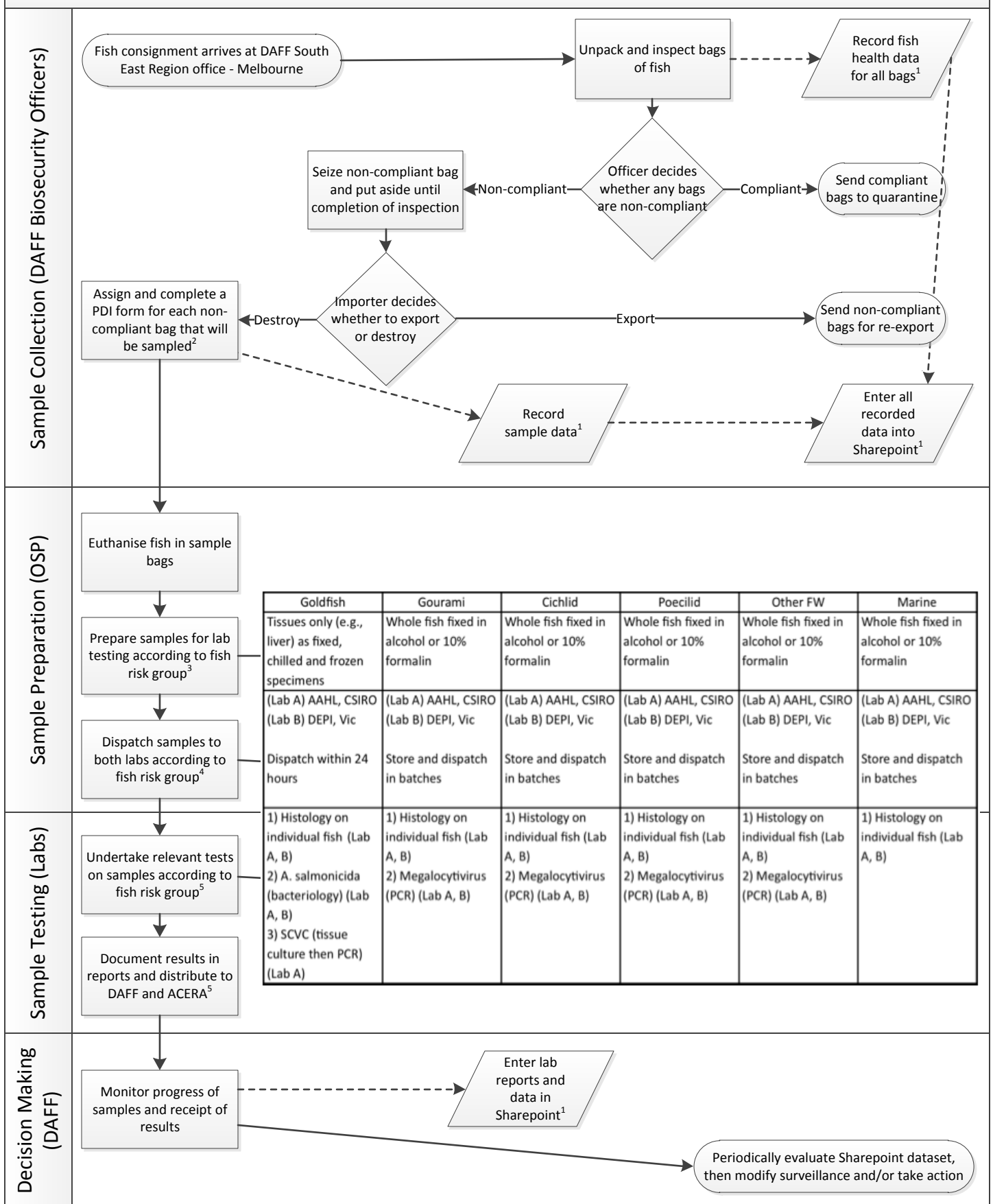
Quarantine Entry (AIMS) Code...   Row ID (from Sheet A)..........   PDI Number...

Reason(s) for Non-compliance of the Bag? [Tick all that apply]	Sign(s) of III Health for Each Sampled Fish? [Tick all that apply]	Fish Sample Label									
		A	B	C	D	E	F	G	H	I	J
☐ 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 ☐ Mixed species ☐ Signs of disease	None	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Loss of colouring or darkening	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Abnormal swimming behaviours, hovering at the surface	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Lethargy or hyper/hypo-activity	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Fin burn/congestion/erosion	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	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	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Body surface abnormalities such as fungal growth, visible external parasites, spots, raised lesions, ulceration, scale protrusion or scale loss	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Do You Think Morbidity was Caused by Transport Stress?	N Y	N Y	N Y	N Y	N Y	N Y	N Y	N Y	N Y	N Y

General Notes...

Figure 3: Overview of Phase 1 of the Ornamental Fish Import Reform Trial

Sample Collection (DAFF Biosecurity Officers)

Fish consignment arrives at DAFF South East Region office - Melbourne → Unpack and inspect bags of fish ⇢ Record fish health data for all bags[1]

Officer decides whether any bags are non-compliant
- Compliant → Send compliant bags to quarantine
- Non-compliant → Seize non-compliant bag and put aside until completion of inspection

Importer decides whether to export or destroy
- Export → Send non-compliant bags for re-export
- Destroy → Assign and complete a PDI form for each non-compliant bag that will be sampled[2]

Assign and complete a PDI form ⇢ Record sample data[1] ⇢ Enter all recorded data into Sharepoint[1]

Sample Preparation (OSP)

Euthanise fish in sample bags

Prepare samples for lab testing according to fish risk group[3]

Dispatch samples to both labs according to fish risk group[4]

Goldfish	Gourami	Cichlid	Poecilid	Other FW	Marine
Tissues only (e.g., liver) as fixed, chilled and frozen specimens	Whole fish fixed in alcohol or 10% formalin	Whole fish fixed in alcohol or 10% formalin	Whole fish fixed in alcohol or 10% formalin	Whole fish fixed in alcohol or 10% formalin	Whole fish fixed in alcohol or 10% formalin
(Lab A) AAHL, CSIRO (Lab B) DEPI, Vic Dispatch within 24 hours	(Lab A) AAHL, CSIRO (Lab B) DEPI, Vic Store and dispatch in batches	(Lab A) AAHL, CSIRO (Lab B) DEPI, Vic Store and dispatch in batches	(Lab A) AAHL, CSIRO (Lab B) DEPI, Vic Store and dispatch in batches	(Lab A) AAHL, CSIRO (Lab B) DEPI, Vic Store and dispatch in batches	(Lab A) AAHL, CSIRO (Lab B) DEPI, Vic Store and dispatch in batches
1) Histology on individual fish (Lab A, B) 2) A. salmonicida (bacteriology) (Lab A, B) 3) SCVC (tissue culture then PCR) (Lab A)	1) Histology on individual fish (Lab A, B) 2) Megalocytivirus (PCR) (Lab A, B)	1) Histology on individual fish (Lab A, B) 2) Megalocytivirus (PCR) (Lab A, B)	1) Histology on individual fish (Lab A, B) 2) Megalocytivirus (PCR) (Lab A, B)	1) Histology on individual fish (Lab A, B) 2) Megalocytivirus (PCR) (Lab A, B)	1) Histology on individual fish (Lab A, B)

Sample Testing (Labs)

Undertake relevant tests on samples according to fish risk group[5]

Document results in reports and distribute to DAFF and ACERA[5]

Decision Making (DAFF)

Monitor progress of samples and receipt of results ⇢ Enter lab reports and data in Sharepoint[1]

→ Periodically evaluate Sharepoint dataset, then modify surveillance and/or take action

Footnotes
1 Refer to Work Instruction A – Data recording.
2 Refer to Work Instruction B – Sample collection.
3 Refer to Work Instruction C – Sample handling and preparation.
4 Refer to Work Instruction D – Samples labelling and dispatch.
5 Refer to Laboratory Procedures – Protocols and testing guidelines.

Figure 4: Sampling and Testing Structure for Fish Samples Collected During Phase 1 of the Trial

Note. In many cases it will not be possible to sample four marine fish because each fish is individually bagged and the number of available samples depends on the number of non-compliant, to-be-destroyed fish of the same species within the same consignment. For this reason, the number of replicates (and the ability to replicate between labs) will vary between consignments for marine fish; i.e., the above diagram is the aspirational sampling structure, not a strict requirement. Furthermore, documentation of marine fish samples will differ slightly from other fish because each sampled fish will have a PDI number.