Australian Government
Department of Agriculture, Fisheries and Forestry

Review of live marine ornamental fish import policy

Draft report

Animal Biosecurity Branch

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Acknowledgement of Country

We acknowledge the Traditional Custodians of Australia and their continuing connection to land and sea, waters, environment and community. We pay our respects to the Traditional Custodians of the lands we live and work on, their culture, and their Elders past and present.

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Summary

The Australian Government Department of Agriculture, Fisheries and Forestry has conducted this risk review to assess the biosecurity risks associated with the import of live marine ornamental fish from all countries.

This risk review considers scientific information, advice from international scientific experts, relevant industry practices and operational practicalities.

Australia currently permits the importation of live marine ornamental fish providing they meet specific import conditions. The conditions include that fish have only been sourced from the wild and have not been bred or hatched on a farm or other premises. This risk review was initiated in response to trade enquiries, new scientific knowledge, and the impacts of the current policy (which only permits import of marine fish harvested from the wild) on the environment, especially tropical reefs. The biosecurity risks associated with the import of all live marine ornamental fish, sourced from the wild and captive bred populations are assessed in this risk review. The biosecurity measures recommended in this review will be the basis for the import conditions and any import permits issued for the import of live marine ornamental fish.

This risk review proposes that live marine ornamental fish, including fish sourced from captive bred populations, can be permitted import into Australia, subject to a range of biosecurity measures. This risk review identified one hazard that requires biosecurity measures to manage the risks in imported marine ornamental fish to a level which achieves Australia's appropriate level of protection.

This draft report contains details of the risk review for each hazard and the proposed biosecurity measures to manage identified risks.

1 Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity system consists of three focus areas for preventing or responding to the incursion of pests and diseases: overseas; at our border and within Australia. Across these three focus areas, the Australian Government Department of Agriculture, Fisheries and Forestry undertakes a range of policy, operational and compliance functions and implements various education, awareness and communication campaigns.

Biosecurity risk cannot be reduced to zero at our border. The success of the national biosecurity system in protecting Australia's environment, economy and way of life relies on the efforts of all parties and is a shared responsibility. The department works across the Commonwealth and with governments, industry, research institutions and community groups to implement improvements across the system to manage biosecurity risk efficiently and effectively.

The risk analysis process is an important part of Australia's biosecurity system. It enables the Australian Government to formally consider the level of biosecurity risk that may be associated with proposals to import goods into Australia. If the biosecurity risks do not achieve Australia's appropriate level of protection (ALOP), biosecurity measures are proposed to reduce the risks to an acceptable level. If the risks cannot be reduced to an acceptable level, the goods will not be imported into Australia until suitable measures are identified.

Successive Australian Governments have maintained a conservative, but not a zero risk, approach to the management of biosecurity risks. This approach is expressed in terms of Australia's ALOP, which is described as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

1.1.1 Australia's appropriate level of protection

As per our international obligations, Australia applies ALOP in a consistent way across all products (that is, aquatic animals and aquatic animal products, terrestrial animals and terrestrial animal products, and plants or plant products). The biosecurity risks associated with imported products are assessed through a science-based process. As unique risk factors and scenarios apply to each product, those risks are managed in different ways to ensure that Australia's ALOP is achieved. They also consider the World Organisation for Animal Health (WOAH) recommendations for managing biosecurity risk associated with the product or live animal. Importantly, biosecurity measures are selected based on whether they reduce risk to a level that achieves Australia's ALOP.

1.2 This risk review

The department undertakes risk analyses using technical and scientific experts in relevant fields and involves consultation with stakeholders at various stages during the process.

Risk analyses may be conducted as a biosecurity import risk analysis (BIRA) or a non-regulated risk analysis. A BIRA is a risk analysis with key steps that are conducted by the department under the Biosecurity Act 2015. A BIRA only occurs in cases where relevant risk management measures have either not been established or exist for a similar good and pest or disease combination, but the

likelihood and/or consequences of entry, establishment or spread of pests or diseases could differ significantly from those previously assessed.

A risk analysis which does not meet the criteria of a BIRA is undertaken as a non-regulated analysis of existing policy. Non-regulated risk analyses include scientific reviews of existing policy (such as this risk review) or import conditions and reviews of biosecurity measures in light of new scientific information. Non-regulated risk analyses are undertaken through an administrative process which is not provided for in law, however they still meet Australia's international rights and obligations. Non-regulated risk analyses use a similar technical methodology as BIRAs. It is important to note that not all non-regulated risk analyses are undertaken as a formal risk review such as this one. Notifications are only given to stakeholders through Biosecurity Advice notices for significant or complex scientific reviews of existing policy. Less significant or complex risk reviews may not be released publicly unless the review determines that changes need to be made to import conditions. Further information about Australia's biosecurity framework is provided in the Biosecurity import risk analysis guidelines 2016.

1.2.1 Background

This risk review is conducted as a non-regulated risk analysis of the existing import conditions and policy, including the *Import risk analysis on live ornamental fish* released in 1999 (Ornamental fish IRA) (AQIS 1999) and the *Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses—final import risk analysis report* (Gourami iridovirus review) (Department of Agriculture 2014).

The current policy for the import of live marine ornamental fish only permits import of species sourced from the wild. This risk review commenced in response to trade enquiries from industry and in recognition of new scientific knowledge, advances in captive breeding of marine ornamental fish, and the impacts of the current policy (which only permits import of marine fish harvested from the wild) on the environment, especially tropical reefs. For this risk review, the department intended to only assess the known biosecurity risks associated with captive bred marine ornamental fish (fish that were spawned, hatched, settled, and grown to juvenile or adult stage in an enclosed system). However, considering changes to biosecurity risks of live marine ornamental fish since the Ornamental fish IRA was released in 1999, the scope was extended to live marine ornamental fish sourced from the wild and captive bred populations. This risk review determines if and how live marine ornamental fish could be imported in a manner that achieves Australia's ALOP.

The biosecurity measures recommended in the final report for this review will be the basis for the import conditions and any import permits issued for the import of live marine ornamental fish.

1.2.2 Scope

The scope of this risk review is live marine ornamental fish currently included on the department's List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A) which have been sourced from all countries and all production systems (i.e. wild-caught and captive bred). A country must be on the *Department approved countries for export of live marine ornamental fish* list before they are considered an 'approved country' for the export of marine ornamental fish to Australia. Approval of countries to export live ornamental fish to Australia is not included in this risk review.

1.2.3 Existing policy

Import policy

The department is responsible for managing the biosecurity risk of live animal imports under the Biosecurity Act 2015. The current import requirements for live marine ornamental fish are on the Australian Biosecurity Import Conditions (BICON) website. Separate import conditions for live freshwater ornamental fish can also be found on BICON. All fish imported under these requirements are to be used for display (ornamental) purposes only.

Live marine ornamental fish exported to Australia require a valid import permit and must be accompanied by an official government health certificate attesting that:

- Only fish listed on the List of permitted live marine ornamental fish suitable for import are included in the consignment, and are documented on an invoice accompanying the consignment.
- The fish in the consignment have been inspected within 7 days prior to export and show no clinical signs of infectious disease or pests.
- The fish were exported from premises approved to export marine fish to Australia by a Department of Agriculture, Fisheries and Forestry recognised competent authority of the exporting country.
- The fish were collected from an area at least 5 kilometres from any fish aquaculture operation and the fish in the consignment have not come into contact with water, equipment or fish associated with farmed foodfish (fish farmed for human consumption including recreational fishing).
- The fish are not sourced from a population associated with any significant infectious disease or pests and there have not been any outbreaks of infectious fish disease or pests in the areas from which the fish have been collected during the 6 months prior to collection.
- The fish are wild-caught and have not been bred or hatched on a farm or other premises.

On-arrival, all marine ornamental fish are subject to visual inspection by the department to ensure:

- they are healthy
- the health certification and invoice are in order
- they are an approved species
- they do not contain non-permitted material, or material of biosecurity concern.

Following visual inspection, all consignments are directed for isolation at an approved arrangement site used for holding aquarium fish (7.1 Aquarium fish) for 7 days. At the end of the quarantine period, the fish are inspected by the department and if found free from clinical signs of pests and diseases they are released from biosecurity control.

The department has procedures in place to approve countries for exporting ornamental fish to Australia, to ensure Australia's import conditions can be met. This includes an assessment of the country in relation to several criteria:

- aquatic animal health status and reporting to the WOAH

- aquatic animal health legislation
- systems for control over certification of live animals
- veterinary and laboratory services
- performance in disease notification
- biosecurity requirements
- disease surveillance, management, and control programs
- general veterinary services capacity.

Domestic arrangements
The Australian Government is responsible for regulating the movement of animals and animal products into and out of Australia. However, the state and territory governments are responsible for animal health and environmental controls within their individual jurisdictions.

1.3 Consultation

On XX Month 2024, the department released Animal Biosecurity Advice 2024-A0X inviting stakeholders to provide comment on this draft report.

During preparation of this risk review, the department sought input from state and territory governments and the ornamental fish industry. The draft report was peer-reviewed by a leading independent expert in ornamental fish diseases.

1.4 Ornamental fish industry

Aquarium keeping is a popular hobby, with millions of enthusiasts worldwide. Aquariums maintained by these enthusiasts often aim to replicate a natural ecosystem and can support multiple species.

1.4.1 Global marine ornamental fish trade

The marine ornamental fish trade is expanding globally, but still relies on harvesting wild fish from tropical coral reef ecosystems to meet demand (Pouil et al. 2020). Generally, marine fish are considered more difficult to grow in aquaculture than freshwater species because of their specific environmental requirements for maintenance and reproduction. Substantially more freshwater ornamental species are grown in aquaculture and at a larger scale of production than marine ornamental fish species.

In the past decade, reef fish aquariums have increased in popularity, increasing the demand for marine fish. In 2015, 20–40 million marine ornamental fish from 1,471 species were estimated to be traded globally (Kumar, Gunasundari & Prakash 2015). In 2022 in the United States, 14.7 million households reported owning aquarium fish of which 2.9 million households owned marine fish (Institute 2023). Because marine ornamental fish are predominantly captured from the wild, particularly from reefs, a new driver for increasing aquaculture production for marine fish is sustainability and environmental protection. In response to the drive for sustainability, countries are beginning to develop legislation to protect marine environments and their aquatic life from collectors and other sources of damage.

Recent research and advances in marine ornamental fish aquaculture has increased the number of marine fish species farmed. In 2019, 398 marine species were reported to have been successfully bred in captivity (CORAL Magazine 2019). Approximately 11.5% of these were commonly available for commercial purchase, 67% were not seen to be available for purchase and the availability of the remainder was variable (CORAL Magazine 2019). However, based on analysis of data on the availability and range of captive breeding, the vast majority of marine ornamental fish are still wild-caught (Pouil et al. 2020).

1.4.2 Ornamental fish trade in Australia

Recent data on the value of the ornamental fish trade in Australia is unavailable. This is because ornamental fish can be traded from multiple pathways to multiple endpoints, including aquarium stores, private collections, wholesalers, commercial breeding facilities and importers, making monitoring of the industry challenging (Millington, Holmes & Balcombe 2022). The most recent estimate of the value of the ornamental fish trade in Australia was A$350 million annually, including both marine and freshwater species (Natural Resource Management Ministerial Council 2006).

Under the conditions put in place by the 1999 Ornamental fish IRA (AQIS 1999), live marine ornamental fish are only permitted import to Australia if they are certified as being sourced from the wild and not captive bred. Captive bred is defined as marine ornamental fish that were spawned, hatched, settled and grown to juvenile or adult stage in an enclosed system. Of those marine species reported to have been successfully bred in captivity and commonly available (CORAL Magazine 2019), 40% are included on the department's *Permitted marine species list*. The Ornamental fish IRA did not consider the biosecurity risks associated with captive bred marine ornamental fish because at that time, less than 1% of ornamental fish imports into Australia were marine and greater than 98% of the global trade in marine ornamental fish was wild-caught (AQIS 1999). As shown in Table 1, from 2020–23 import of marine ornamental fish into Australia has only increased to 1.2% of total ornamental fish imports. The major exporters of marine ornamental fish were Indonesia (74.7%), Philippines (12%), and Sri Lanka (7%), with the remaining 6.3% spread across New Caledonia, Fiji, Singapore, the United States of America, Kenya and Malaysia (source: Department of Agriculture, Fisheries and Forestry databases of live ornamental fish imports). The estimated value of imported live ornamental fish in 2021–22 was A$6.7 million but this was for both marine and freshwater species (Tuynman et al. 2023).

Table 1 Volume of live ornamental fish imported into Australia across 2020–23

	Volume (individuals) imported per financial year		
Group	**2020–21**	**2021–22**	**2022–23**
Marine species [a]	125,962	143,112	146,980
Megalocytivirus-susceptible freshwater species [b]	3,231,301	3,066,101	3,009,797
Other freshwater species [c]	6,193,994	6,166,034	5,629,363
Goldfish [d]	2,558,175	2,047,355	1,881,940
Total	**12,109,432**	**11,422,602**	**10,688,080**

a Marine fish species included on the department's List of permitted live marine ornamental fish suitable for import. **b** Species of gouramis, cichlids, poecilids, paradise fish and bettas included on the department's List of permitted live freshwater fish suitable for import. **c** Freshwater fish species, other than gouramis, cichlids, poecilids, paradise fish and

bettas, included on the department's List of permitted live freshwater fish suitable for import. **d** *Carassius auratus*.
Source: Department of Agriculture, Fisheries and Forestry databases of live ornamental fish imports.

Australian ornamental fish aquaculture

In 2021–22, the estimated aquaculture production of ornamental fish was A$289,000 in New South Wales (volume not available), A$773,000 in Victoria (volume not available), A$305,000 in Western Australia (volume not available). Production also occurred in the Northern territory and Queensland, although value and volume were not available (Tuynman et al. 2023). In addition to commercial breeders, ornamental fish are also bred by wholesalers, retailers and the hobby sector, which is difficult to quantify.

1.5 Other fish industries

1.5.1 Fish aquaculture and fisheries

The gross value of Australia's fish production in 2021–22 was estimated at A$1.97 billion from a total weight of 230,929 tonnes (Tuynman et al. 2023). The aquaculture industry produced 100,198 tonnes valued at A$1.40 billion while wild-caught fisheries produced 135,673 tonnes with an estimated value of A$594 million (Tuynman et al. 2023). Key cultured and wild-caught fish species production values and volumes are presented in Table 2.

Fisheries and aquaculture based in state and territory waters produced 88% of total fisheries production for 2021–22 (Tuynman et al. 2023). Management of these industries is the responsibility of the various state and territory governments under their relevant fisheries legislation. Under the Australian Government *Fisheries Management Act 1991*, the Australian Fisheries Management Authority is responsible for the development and administration of management plans for marine fisheries in Commonwealth waters (AFMA 2022).

Table 2 Top 3 aquaculture and fisheries fish production values and volumes 2021–22

Industry	Value (A$)	Volume (tonnes)
Salmonid (aquaculture)	1.15 billion	81,279
Southern bluefin tuna (aquaculture)	110 million	8,322
Barramundi (aquaculture)	61 million	4,741
Tuna (wild-caught)	61 million	8,597
Sardines (wild-caught)	35 million	49,738
Whitings (wild-caught)	26 million	3,041

Source: Australian Bureau of Agricultural and Resource Economics and Sciences Australian Fisheries and Aquaculture Statistics 2022 (Tuynman et al. 2023).

Australia's trade in fish

Fish imports

The value of imported finfish, shark and ray products in 2021–22 was A$1.20 billion (156,965 tonnes) (Tuynman et al. 2023). The major countries exporting fishery products to Australia in 2021–22 were Vietnam, Thailand, China and New Zealand (Tuynman et al. 2023). No live imports of fish for commercial aquaculture or human consumption have been permitted into Australia for over 50 years. However, Australia's largest fish aquaculture industry is based on *Salmo salar* (Atlantic salmon), first introduced from the United Kingdom in 1864 (Clements 1988).

Fish exports

In 2021–22, Australia exported 26,537 tonnes of salmonids (A$417 million) and 10,199 tonnes of tuna (A$135 million). An additional 10,459 tonnes of finfish, shark and ray products (from both aquaculture and wild fisheries sectors) valued at A$96.6 million were exported (Tuynman et al. 2023). The major export destinations for Australian fisheries and aquaculture products were China, Hong Kong, Japan, United States of America and Vietnam (Tuynman et al. 2023). In 2021–22, A$2.3 million of live ornamental fish were also exported (volume not available) (Tuynman et al. 2023).

1.5.2 Recreational fishing

Recreational fishing is a popular activity that contributes economic and social benefits, particularly in regional areas (McManus et al. 2011; Moore et al. 2023). The most recent national social and economic survey of recreational fishers estimates that a total of 4.2 million or 1 in 5 adult Australians participate in recreational fishing each year (a participation rate of 21%), improving wellbeing and contributing 100,000 jobs and $11 billion to the Australian economy (Moore et al. 2023).

1.6 Risk review process

Risk review is not defined or described in the WOAH *Aquatic animal health code* (WOAH Code), however risk analysts recognise risk review as an essential component of the risk analysis process (Barry 2007; FSA 2006; Purdy 2010).

Australia applies a process of risk review to the biosecurity risks associated with the importation of an animal commodity (animal product or live animal) for which current biosecurity measures exist.

Sources of information drawn on for this risk review include (this list is not exhaustive):

- the WOAH Code (WOAH 2023a)
- the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH 2023g)
- the Ornamental fish IRA (AQIS 1999)
- the Gourami iridovirus review (Department of Agriculture 2014)
- current requirements for importation of live ornamental fish into Australia
- relevant scientific information
- expert opinion
- policies adopted by other countries for the importation of live ornamental fish.

Risk, defined by the WOAH Code as ‘the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health’ (WOAH 2023a), is dynamic in nature and changes with time. Consequently, regular review of risk should be undertaken.

Risk review differs from the monitoring and review component of risk management, as described in the WOAH Code, in that each component of the risk analysis process (hazard identification, risk assessment and risk management) is reviewed under the risk review process. Based on updated scientific information, if it is identified that there has been a change (either an increase or a

decrease) in the biosecurity risk associated with a live animal or animal products currently imported into Australia, biosecurity measures can be revised accordingly.

1.6.1 Review of hazard identification

The WOAH Code (Article 2.1.2) describes hazard identification as a classification step done to identify potential hazards that may be associated with the importation of a commodity (WOAH 2023a).

In accordance with the WOAH Code (WOAH 2023a), a pathogenic agent was considered a potential hazard relevant to the importation of marine ornamental fish (included on the department's *Permitted marine species list*) if it was assessed to be:

- 'appropriate' to the species to be imported, or from which the commodity is derived
- present in the exporting country
- able to potentially produce adverse consequences in the importing country
- not present in the importing country, and if present, associated with a listed disease, or subject to control or eradication measures.

Where evidence for the inclusion or exclusion of a pathogenic agent was equivocal, a judgement was made based on the strength of the available evidence to implicate marine ornamental fish included on the department's *Permitted marine species list* in disease transmission.

1.6.2 Review of risk assessment

A review of risk factors relevant to the entry, exposure and consequence assessment was conducted for each hazard retained for risk review. If definitive information on risk factors was not found through literature review or contact with relevant experts, any uncertainties were identified and documented.

Based on the information reviewed, a conclusion was reached for each hazard about whether a significant change in biosecurity risk had occurred that was relevant to the importation of marine ornamental fish into Australia. Assumptions and judgements that were made in drawing conclusions for each hazard were documented in the relevant risk review chapters.

The likelihood that a hazard would enter Australia, and the likelihood of exposure of susceptible animals to the hazard, were determined through an 'entry assessment' and 'exposure assessment', respectively. The 'likelihood of establishment and spread' and the 'adverse impacts', were determined through a 'consequence assessment'. The risk assessment for an identified hazard concluded with 'risk estimation'.

1.6.3 Review of risk management

The WOAH Code (chapter 2.1) (WOAH 2023a) divides risk management into four components:

- risk evaluation
- option evaluation
- implementation
- monitoring and review.

Risk evaluation
Risk evaluation is the process of comparing the risk estimated in the risk assessment with the WOAH member's ALOP.

Risk evaluation during this risk review was based on the conclusions drawn from the risk assessments conducted for each hazard. A judgement was made to determine whether risk management was warranted to achieve Australia's ALOP.

Option evaluation
Option evaluation ultimately results in selection of a biosecurity measure which will reduce the risk associated with the importation of a product to a level which achieves the WOAH member country's ALOP. The process of option evaluation includes considering the efficacy and feasibility of the biosecurity measure.

The efficacy is the degree to which an option reduces the likelihood and/or magnitude of adverse health and economic consequences. Evaluating the efficacy of the options selected is an iterative process that involves their incorporation into the risk assessment and then comparing the resulting level of risk with that considered acceptable. The evaluation for feasibility normally focuses on technical, operational, and economic factors affecting implementation of the risk management options.

Implementation
Implementation is the process of following through with the risk management decision and ensuring that the biosecurity measures are in place.

Monitoring and review
Monitoring and review are the ongoing processes by which biosecurity measures are continually audited. This ensures that they are achieving the results intended.

The department is responsible for monitoring and reviewing any applied biosecurity measures to enable the safe importation of live ornamental fish.

1.6.4 Risk communication

Risk communication is defined in the WOAH Code as:

> the interactive transmission and exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions among risk assessors, risk managers, risk communicators, the general public and other interested parties (WOAH 2023a).

In conducting risk analyses and policy reviews, the department consults with the Department of Health to ensure that public health considerations are included in the development of Australia's animal biosecurity policies. Consultation with external stakeholders is a standard procedure for all import risk analyses and risk reviews. Consultation on this risk review enables stakeholder feedback on draft conclusions and recommendations about Australia's biosecurity policies. Peer review is an essential component of risk communication to obtain a scientific critique and to ensure that the data, information, methods and assumptions are the best available.

When undertaking this risk review, the Aquatic biosecurity risk assessment unit (ABRA) has been the first point of contact for all related questions. ABRA has provided periodic updates on this risk review since early-2023 and will remain to do until the release of the final report.

2 Method

This chapter provides the risk assessment methodology and the general considerations and key assumptions used by the department when undertaking this risk review.

The World Organisation for Animal Health (WOAH) Aquatic animal health code (WOAH Code) describes 'General obligations related to certification' in chapter 5.1 (WOAH 2023g).

The WOAH Code states in Article 5.1.2. (WOAH 2023g) that:

> The import requirements included in the international aquatic animal health certificate should assure that commodities introduced into the importing country comply with WOAH standards. Importing countries should align their requirements with the recommendations in the relevant standards of the WOAH. If there are no such recommendations or if the country chooses a level of protection requiring measures more stringent than the standards of the WOAH, these should be based on an import risk analysis conducted in accordance with chapter 2.1.

Article 5.1.2 (WOAH 2023g) further states that:

> The international aquatic animal health certificate should not include measures against pathogenic agents or diseases that are not WOAH listed, unless the importing country has demonstrated through an import risk analysis, carried out in accordance with Section 2, that the pathogenic agent or disease poses a significant risk to the importing country.

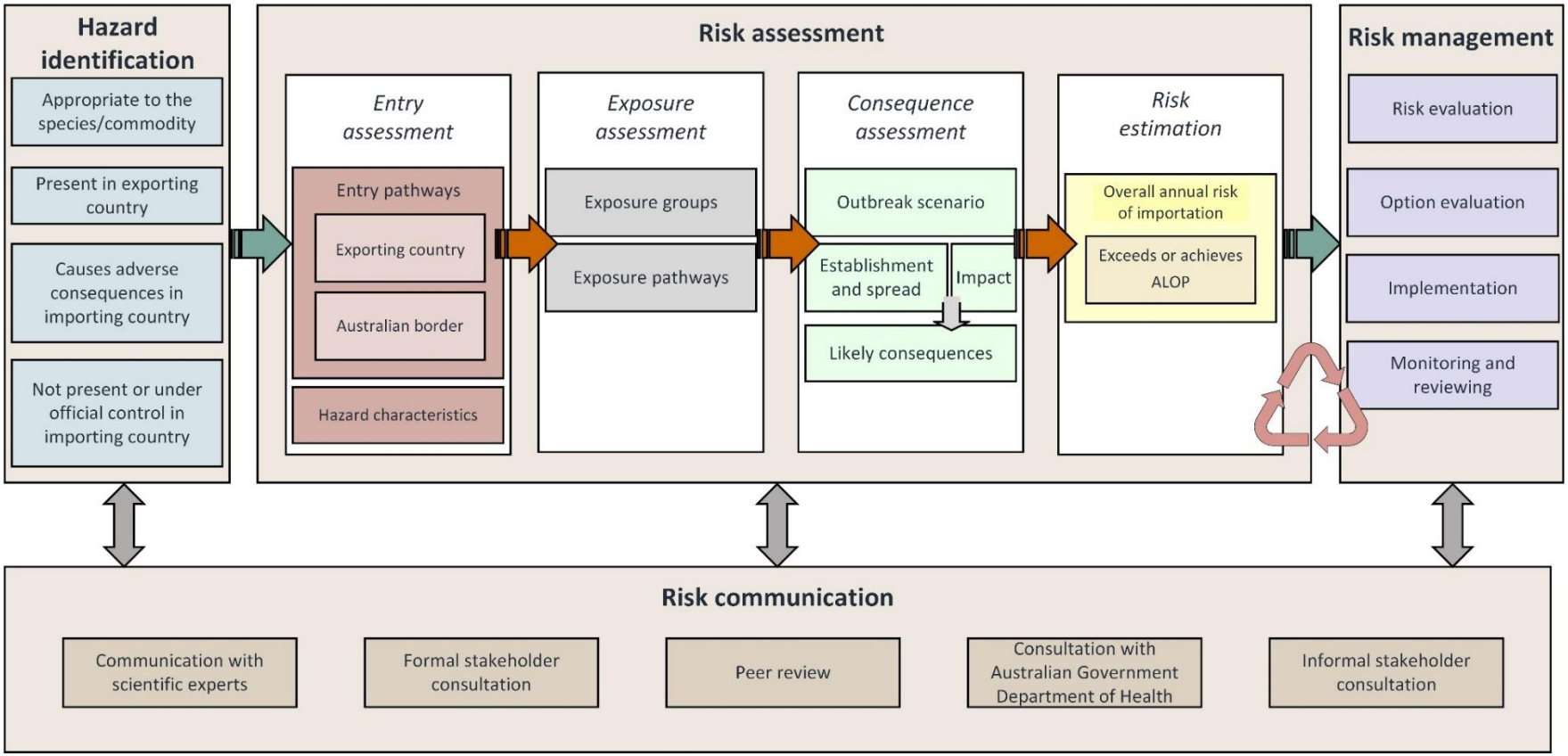
There are four components of risk analysis as described in chapter 2.1 of the WOAH Code (WOAH 2023g):

- hazard identification
- risk assessment (entry, exposure and consequence assessments and risk estimation)
- risk management
- risk communication.

Hazard identification, risk assessment and risk management are sequential steps within a risk analysis. Risk communication is an ongoing process and includes both formal and informal consultation with stakeholders.

Figure 1 shows the components of the import risk analysis process and the steps within the risk assessment process.

Figure 1 Components of the import risk analysis process and the steps within the risk assessment process



The import risk analysis process comprises of four components. **Hazard identification** the first component involves identifying the pathogenic agents associated with the importation of a commodity, that could potentially produce adverse consequences. Risk assessment the second component, is completed for each hazard. It estimates the risks associated with the hazard and is comprised of four steps. **Entry assessment** describes the pathway(s) for the introduction of the hazard into Australia and estimates the likelihood of entry for that hazard. **Exposure assessment** determines, for each hazard, the likelihood that a susceptible species in Australia would be exposed to the agent via imported live marine ornamental fish or associated wastes. **Consequence assessment** describes the potential consequences of a given exposure and estimates the probability of them occurring. **Risk estimation** integrates likelihood of entry and exposure and likely consequences to obtain the overall annual risk associated with a hazard. **Risk management** the third component of risk analysis is the process of identifying, selecting and implementing measures that can be applied to reduce the level of risk of a hazard, while at the same time, ensuring that negative effects on trade are minimised. **Risk communication** the fourth component of risk analysis, is an ongoing process and includes both formal and informal consultation with stakeholders and peer review.

2.1 Evaluating and reporting likelihoods

Likelihood estimations made in this assessment were based on information available in the scientific literature, unpublished data, as well as the expert judgement of the department and other experts.

This risk review used a qualitative approach. The likelihood of entry, exposure, and establishment and spread was evaluated and reported using qualitative likelihood descriptors as described in Table 3.

Table 3 Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition
High	The event would be very likely to occur
Moderate	The event would occur with an even probability
Low	The event would be unlikely to occur
Very low	The event would be very unlikely to occur
Extremely low	The event would be extremely unlikely to occur
Negligible	The event would almost certainly not occur

Estimating the likelihoods associated with entry, exposure and establishment and spread involved examining the various factors that influence those likelihoods.

Entry and exposure likelihood estimations consider the likelihood of the event occurring over a one-year period. This is considered a sufficient period to enable evaluation of seasonal effects, but not so long as to incorporate effects that may be associated with significant changes in disease factors, host factors or factors associated with trade. Entry and exposure assessments for each hazard considered the expected annual volume of trade in the commodity. The previous year's trade was the basis for the expected annual volume of trade. There were no changes in import conditions in the 2021–22 financial year to consider when using this data to estimate the expected volume of trade (refer BICON).

2.2 Entry assessment

The entry assessment determines the annual likelihood of entry into Australia of each hazard. In this risk review, consideration is given to the single-entry scenario which is the importation (from all countries) into Australia of live marine ornamental fish.

Fish are placed in polythene bags approximately one third filled with seawater in preparation for transport. The bags are inflated with pure oxygen (two thirds), sealed with rubber bands or clips and placed in polystyrene boxes or cartons fitted with a plastic lining and then sealed. There may be one or more bags per box, but each bag is required to contain only one species of fish. Each box or carton is then labelled and individually identified.

Once packaged, the fish are transported to wholesalers or export distribution centres where the fish may be unpacked and held in a holding facility for conditioning/stabilisation to ensure they are fit for export. An export distribution centre may be located in a different country from where the fish were farmed or collected from the wild. Any fish showing clinical signs of disease or visible presence of parasites are treated while being acclimatised in the holding facility or disposed. Acclimatization is

the process of slowly introducing the fish to different quality water to allow physiological adjustments to occur gradually over time.

Once the bags are unloaded at the final packaging room, the water is again changed and the bags re-oxygenated. Fish for export are packaged as described earlier for transportation in sealed polythene bags in insulated cardboard boxes.

In the wholesale or export distribution centre holding facilities, fish from different sources may again be mixed prior to export to meet customer orders. Cross contamination of fish from different batches may occur in these facilities due to inadequate cleaning and disinfection of equipment between batches.

In export distribution centres, ornamental fish destined for Australia are subject to quarantine isolation and visual inspection (in accordance with current import conditions) and exported to Australia accompanied by a health certificate provided by the competent authority of the exporting country.

On arrival in Australia, live marine ornamental fish are inspected by the Department of Agriculture, Fisheries and Forestry to ensure that they are healthy, the health certification and invoice is in order, they are an approved species and they do not contain non-permitted material or material of biosecurity concern. All marine ornamental fish are then directed for isolation by the department at an ornamental fish approved arrangement site (AA site) for 7 days. At the end of the quarantine period, the fish are inspected at the AA site by the department and must be found free from clinical signs of pest and disease before they are released from biosecurity control.

2.2.1 Key considerations of the entry assessment

Several key factors were considered when determining the likelihood of viable and infective hazards being present in live marine ornamental fish imported into Australia, including the:

- Volume and species of live marine ornamental fish imported into Australia.
  - Approximately 65,000–80,000 individual marine ornamental fish are imported annually.
  - Only live marine fish species included on the department's List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A) are permitted import.
- Biological characteristics of each hazard, including the:
  - Life cycle of the hazard.
  - Host range of the hazard.
  - Expected prevalence of the hazard in the imported fish.
  - Whether there is carrier status, subclinical presentation, or obvious gross clinical signs of infection.
- Commodity factors, including:
  - The likelihood of the hazard being present in an infective form in the fish (or associated wastes) being imported.

Following review of the Ornamental fish IRA and the Gourami iridovirus review, this risk review made assumptions when estimating the likelihood of entry for each hazard, including:

- The current pre-export and post-arrival risk management measures for the importation of live marine ornamental fish (with the exception of the requirement that they are wild-caught) are in place at the time of import.
- The hazards are present in all source countries.
  - The absence of a disease agent from a region is an important consideration in an entry assessment. However, as the scope of this risk review includes importation of live marine ornamental fish from all countries, the entry assessment assumes that the hazards are present in all source countries. Country, compartment or zone freedom from hazards may be considered as a biosecurity measure if necessary.
- Pre-export inspection typically focuses on the physical appearance of the fish to be exported. It is unlikely that fish which are sub-clinically infected or with mild gross signs will be identified and removed, and therefore this cannot be relied upon to reduce entry likelihood.
  - Generally, ornamental fish are checked for visible external parasites and clinical signs of disease prior to harvesting or following collection from the wild, and only visibly healthy fish are moved to holding tanks for sorting (e.g. size and male/female), counting and export.
    - Fish showing clinical signs of disease may undergo treatment and be held on the farm until they recover and are fit for transportation.
  - Apparently healthy fish may carry disease agents without showing clinical signs of disease.
    - The likelihood of shipments carrying subclinically infected fish would depend on the prevalence of disease in the source population and the nature of the hazard in the species being imported.
  - Fish that are obviously diseased at any point during the supply-chain (pre-export, on-arrival or post-quarantine) are unlikely to be supplied further as poor-quality shipments would lead to loss of business.

2.2.2 Estimation of likelihood of entry

The entry assessments considered the above factors and estimated the annual likelihood of each hazard entering Australia in imported live marine ornamental fish. The entry assessment used the qualitative likelihood descriptors described in Table 3.

The outcome of the entry assessment was the annual **likelihood of entry** (LR) into Australia of the hazard.

2.3 Exposure assessment

The exposure assessment determines the likelihood of direct exposure of a susceptible population (exposure group) in Australia to each hazard via potentially infected live imported marine ornamental fish (or associated wastes). All estimates of the likelihood of exposure assume the hazard is present in the live marine ornamental fish at the time of arrival in Australia. Once released from post-arrival quarantine, most imported live marine ornamental fish are expected to be sent to

wholesale facilities and generally sold to retail shops such as aquarium or pet stores. In these stores, imported live marine ornamental fish may be mixed with Australian bred or wild-caught fish. From the retailer, most would be on-sold for display purposes in home aquariums, a small number may end up as broodstock for breeding purposes or as display in public aquariums and some may be used as feed/bait for other fish or released directly into the wild (deliberately or inadvertently).

The factors considered when estimating the likelihood of an exposure group directly encountering a hazard, for each major exposure pathway, included the:

- Likelihood of live imported marine ornamental fish entering the general environment of the exposure groups.
- Amount of infectious hazard in the live imported marine ornamental fish (or associated wastes) at point of exposure.
- Likelihood of contact between susceptible host animals and live imported marine ornamental fish (or associated wastes).

2.3.1 Identification of exposure groups

Following review of the Ornamental fish IRA and the Gourami iridovirus review, this risk review identified three exposure groups based on potential end uses for imported live ornamental fish. The exposure groups are:

- Australian ornamental fish populations (susceptible species of ornamental fish in Australia that are not under biosecurity control).
- Farmed foodfish populations (susceptible species of fish farmed for human consumption).
- Wild fish populations (susceptible species of fish in the wild).

The Australian ornamental fish exposure group comprises ornamental fish that are not under biosecurity control, which includes ornamental fish within commercial aquaculture facilities, wholesale facilities, pet stores, public aquariums and home aquariums. Imported ornamental fish within approved arrangement sites are not encompassed in this exposure group because they are the imported commodity. The farmed foodfish exposure group includes fish grown for human consumption (or recreational fishing) in ponds, raceways, cages and tanks, as well as foodfish species kept in research facilities and government hatcheries. The wild fish exposure group are susceptible fish located in natural waterways.

The Ornamental fish IRA and the Gourami iridovirus review both identified that most imported ornamental fish are destined for home aquaria (AQIS 1999; Department of Agriculture 2014). There is no evidence to suggest this assumption is not valid now, and in the case of imported live marine ornamental fish, it is expected this is even more so the case. Marine ornamental fish are high value, and have advanced husbandry needs and specific environmental requirements, meaning the likelihood of their diversion, and subsequent survival, to other uses is less compared to freshwater ornamental species (Groover, DiMaggio & Cassiano 2020).

2.3.2 Identification of exposure pathways

Major exposure pathways are those that are direct and that have a high probability of completion. They contribute substantially to the total likelihood of exposure occurring. In comparison, minor

exposure pathways are unlikely to add appreciably to the overall risk and any biosecurity measures that are necessary to mitigate the major exposure pathways would also likely be sufficient to manage the minor pathways. Therefore, the minor exposure pathways are not considered further in this risk review.

Following review of the Ornamental fish IRA and the Gourami iridovirus review, this risk review identified three major pathways as substantially contributing to the total risk:

- Direct release of imported live marine ornamental fish into the Australian ornamental fish population.
- Direct release of imported live marine ornamental fish into natural waterways.
- Direct release of imported live marine ornamental fish into the farmed foodfish population.

Several minor exposure pathways were also identified. These exposure pathways have a lower probability of completion because inactivation of the hazard occurs before potential exposure. Other potential exposure pathways are outside the scope of this risk review. When the Ornamental fish IRA was released in 1999, diversion of imported ornamental fish for use as broodstock or fingerlings for foodfish aquaculture operations was assumed not to occur (AQIS 1999). However, in 2004 it was determined that advances in the aquaculture industry had the potential to impact the source and end-use, and therefore potential exposure pathways, of imported *Cromileptes altivelis* (barramundi cod). As a result, the import of live barramundi cod was suspended.

The biosecurity risks associated with live fish being imported for farmed foodfish aquaculture purposes would need to be considered through a separate risk analysis and was considered outside the scope of this risk review. However, as part of this risk review, the department has reviewed the interim conditions established in 2004 suspending the importation of barramundi cod for ornamental purposes, and also considered if any other live marine ornamental fish have the potential to be used as broodstock or fingerlings for the aquaculture industry (refer Appendix B). Therefore, this pathway is not considered as a major or minor pathway.

Another potential exposure pathway is the approved arrangement (AA) sites for holding imported ornamental fish. Approved arrangements set out the requirements for undertaking activities. These arrangements allow operators to manage biosecurity risks and/or perform the documentary assessment of goods in accordance with departmental requirements, using their own sites, facilities, equipment and people, and without constant supervision by the department and with occasional compliance monitoring or auditing. Some requirements are specific to the class of AA, and some apply across multiple classes. The type of activity taking place in the AA and the associated biosecurity risks determines the class of AA. Class 7.1 – Aquarium fish is the AA site used for holding live freshwater and marine ornamental fish that are subject to biosecurity control. This pathway was not considered a major or minor pathway because the Class 7.1 requirements ensure management of all associated biosecurity risks, including disposal of wastewater and solids.

Table 4 provides an overview of the major and minor exposure pathways and the key points considered for each, along with the relevant direct exposure group(s) for each pathway. Figure 2 depicts the major and minor exposure pathways by which the three exposure groups could be exposed to imported marine ornamental fish in Australia.

2.3.3 Estimation of partial likelihood of exposure

The likelihood that each exposure group would be exposed to a hazard through contact with imported marine ornamental fish is the partial likelihood of exposure (PLE).

The outcome of the exposure assessment was an estimation of the PLE for each exposure group (described using the nomenclature in Table 3).

Table 4 Key considerations for the major and minor exposure pathways for imported live marine ornamental fish

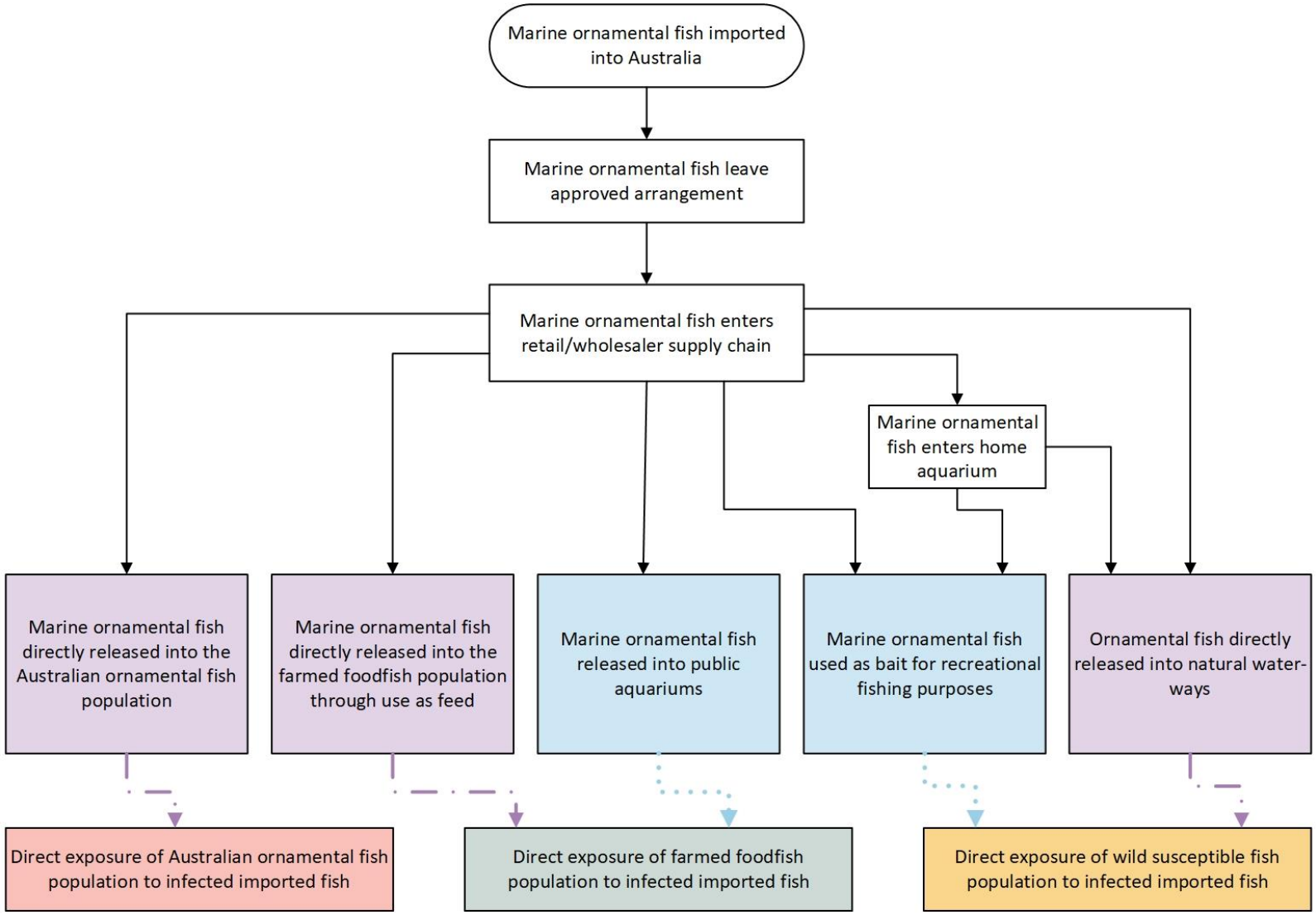
Significance	Pathway	Direct exposure group(s)	Key considerations
Major	1. Direct release of live marine ornamental fish into the Australian ornamental fish population	• Australian ornamental fish	• Australia's ornamental fish population is the most likely exposure group to encounter imported live marine ornamental fish. • Imported live marine ornamental fish may be mixed with domestically produced ornamental fish at wholesale or retail centres or in public or home aquariums resulting in direct exposure of Australian ornamental fish populations to potentially infected imported live marine ornamental fish. In a survey of Australia's marine ornamental supply chain, approximately 83% of Australian marine aquarium businesses and 86% of hobbyists reported mixing imported species in the same aquarium or system with native Australian species (Erickson 2017). • A small number of imported live marine ornamental fish may be used as broodstock for commercial and 'backyard' breeders. If infected, this may result in amplification of diseases and exposure of the Australian ornamental fish population. • Live marine ornamental fish species that are subclinically infected are likely to avoid detection and therefore shed the disease agent into the environment over a prolonged period increasing the exposure likelihood (Hershberger et al. 2010a; Rimmer et al. 2016). In comparison, clinically diseased fish would be expected to be identified and removed from the population out of commercial interests to prevent exposure of naïve fish. • Imported live marine ornamental fish are unlikely to be used as feeder fish for Australian ornamental fish populations because of their cost (Department of Agriculture 2014; Groover, DiMaggio & Cassiano 2020).
	2. Direct release of imported live marine ornamental fish into natural waterways	• Wild fish • Farmed foodfish	• Wild susceptible fish may be directly exposed to imported live marine ornamental fish that are deliberately (e.g. release of sick, dead or unwanted fish) or accidentally (e.g. release of aquarium water) released into the environment. This pathway considers the exposure of fish in natural waters via direct release of imported live marine ornamental fish and disposal of wastewater and/or dead fish into the environment. • The likelihood of release of marine ornamental organisms from the Australian marine ornamental supply chain to the ocean was calculated to be 1.838%, which was determined to represent a probability of an "occasional" release of marine ornamental fish (Erickson 2017). – >94% (hobbyists) and >92% (businesses) never release living marine organisms they no longer want into the ocean. – >98% (hobbyists) and 100% (businesses) never release sick marine organisms into the ocean. – >98% (hobbyists) and 100% (businesses) never release dead marine organisms into the ocean (Erickson 2017). • Traditional and religious practices of 'live release', including Buddhist fang sheng and Tibetan Tsethar (also known as mercy release or prayer animal release) may represent a path for wild susceptible species to be directly exposed to imported live marine ornamental fish. These practices entail the release into the wild of captive animals, particularly those destined for slaughter (Everard et al. 2019; Liu, McGarrity & Li 2012). – Anecdotal evidence suggests the purchase and release of aquarium species for live release was not uncommon in Australia (Lintermans 2004).

Significance	Pathway	Direct exposure group(s)	Key considerations
			• In addition, there is a report of SCUBA divers releasing aquarium-purchased sharks and other large fish into coastal reefs off Singapore's southern islands. (Jaafar et al. 2012). • Live marine ornamental fish could escape from earthen or ground-level ponds (either grow-out ponds in breeding facilities or hobbyists' garden ponds) near or with a direct connection to natural waters, because of vandalism, or accidental or inadvertent breakdown in holding systems (e.g. during floods). – This is considered less of a risk for marine fish than freshwater fish as it is not expected that marine fish would be bred under such situations in Australia. • Reports on spread of diseases due to trade in marine ornamental fish were not found. • Live marine ornamental fish released into the wild are less likely than freshwater ornamental fish to transmit disease to wild susceptible populations due to the significantly greater dilution factor associated with introduction of infectious fish or tissues into seas or ocean environments. In marine settings, water can transmit disease agents between hydrodynamically connected populations. Depending upon the biological characteristics of the hazard, such as the infectious dose, the ability of the hazard to survive outside the host and environmental stability, infection can be initiated in a naïve population without the presence of an infected host (Kough et al. 2014). However, in general these opportunities may be short-lived as hazards must travel on the prevailing currents to reach susceptible populations within a biologically appropriate time and dose, and under appropriate environmental conditions. • It is unlikely that infected live marine ornamental fish released into natural waters will survive for a prolonged period such that they are able to spread hazards. This is because of their expected environmental sensitivity to factors such as salinity and temperature, also their establishment in the wild from a single release is less likely. Temperature is one of the most important environmental factors for fish. It plays an important role in energetics, growth, reproduction, and distribution of fish (Angilletta Jr. et al. 2006). It is recommended that the temperature a tropical marine aquarium should be kept at is 24–28°C (Perry 2023). – Although there are publications recording ornamental trade as a pathway for the accidental introduction of non-indigenous organisms to natural habitats (Jaafar et al. 2012), these have been primarily associated with hardy estuarine species. There was only one example of a marine ornamental fish establishing outside its natural range found. The lionfish (*Pterois volitans* and *Pterois miles*), a marine ornamental fish native to the Indo-Pacific, are reported to have been introduced to the Atlantic Coast of the United States, most likely through release from aquaria, and their distribution expanded into the Caribbean Sea (Blakeway et al. 2022). However, the lionfish expansion and sustained population levels in invaded ranges has been attributed to unique biological characteristics including rapid growth, early sexual maturity, frequent spawning capabilities, physiological tolerances to a breadth of habitat and environmental conditions, disease resistance and lack natural predators (Blakeway et al. 2022). Also, the Mayan cichlid *Cichlosoma urophthalmus,* an ornamental fish native to Central America, has established populations in estuarine rivers and coastal areas in Singapore, where the marine aquaria hobby is very popular (Jaafar et al. 2012). Also, the common molly *Poecilia sphenops*, native to Central and South America and sold as live food for predatory fish in the aquarium trade, is now common in estuarine canals and mangrove streams in Singapore (Jaafar et al. 2012).

Significance	Pathway	Direct exposure group(s)	Key considerations
			• Any sick or dead fish released into natural waterways are likely to be quickly consumed. • The host range will play a significant role in determining the likelihood of exposure of a susceptible species to an imported live marine ornamental fish. For those hazards with a narrow host range, and limited distribution of the susceptible species, exposure is unlikely. The converse is true for those hazards with wide host ranges. • Fish farmed in open cages around areas where live imported marine ornamental fish are deliberately released may be directly exposed. – This is considered unlikely given most farmed fish would not be in the vicinity of the shore where imported live marine ornamental fish would be released. It is also unlikely that individual live marine ornamental fish released on the shore could make their way to a fish cage.
	3. Direct release of imported live marine ornamental fish into the farmed foodfish population	• Farmed foodfish	• Farmed foodfish could be exposed to disease by being fed live or dead marine ornamental fish. – An outbreak of an infectious spleen and kidney necrosis virus (ISKNV) genotype in farmed *Maccullochella peelii* (Murray cod) in 2003 (Go et al. 2006; Lancaster, Williamson & Schroen 2003) identified numerous possible vectors for infection, including infected aquarium fish being fed to broodstock. The virus was subsequently eradicated (Department of Agriculture 2014). – The identification of a potential interface between farmed foodfish and ornamental specimens resulted in the inclusion of this exposure pathway in the Gourami iridovirus review. • Feeding imported marine ornamental fish to farmed foodfish is assumed to be very rare because of their high cost, the large volume that would be required to feed foodfish and the specific nutritional profile that farmed foodfish require.
Minor	1. Disposal of solids and liquid waste from approved arrangement (AA) marine ornamental fish facilities	• Wild fish	• Live marine ornamental fish are required to be kept in an AA on-arrival in Australia. • AA sites utilised for this purpose are required to manage all associated biosecurity risks, including solid waste disposal and water disposal.
	2. Public aquariums	• Wild fish	• Only a very small number of live marine ornamental fish go on display in public aquariums (O'Sullivan, Clark & Morison 2008. • Although a direct deposit of effluent water into the sea by public aquariums may pose a risk, this may be mitigated by the dilution factor of the sea, low stocking densities and treatment of the water by the public aquariums, if such procedures are in place.
	3. Use of fish as bait for recreational fishing purposes	• Wild fish • Farmed foodfish	• Given the high value of imported marine ornamental fish, this is not considered a likely exposure pathway.

Significance: Major exposure pathway that is direct, has a high probability of completion, and contributes substantially to the total likelihood of exposure occurring. **Minor** exposure pathway that has a much lower probability of completion because inactivation of the hazard occurs before potential exposure or because it involves only indirect exposure of the aquatic environment. **Not considered further** exposure pathway has a negligible probability of completion. See Appendix B for consideration of this exposure pathway

Figure 2 Potential exposure pathways of susceptible populations in Australia to imported marine ornamental fish



Major exposure pathways that substantially contribute to total risk are purple boxes and purple dash-dot-dash lines. Minor exposure pathways are blue boxes and blue dotted lines.

2.4 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure (PALEE) is the likelihood that there would be one or more host exposure events over a period of one year. This likelihood was determined for each of the three exposure groups. The PALEE for each exposure group was calculated by combining the likelihood of entry (LR) (see section 2.2.2 Estimation of likelihood of entry) and the corresponding partial likelihood of exposure (PLE) (see section 2.3.3 Estimation of partial likelihood of exposure) using the matrix for combining descriptive likelihoods (see Figure 3).

Figure 3 Matrix for determining the partial annual likelihood of entry and exposure

Likelihood of entry (LR)	Partial likelihood of exposure (PLE)					
	High	Moderate	Low	Very low	Extremely low	Negligible
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate	Moderate	Low	Low	Very low	Extremely low	Negligible
Low	Low	Low	Very low	Very low	Extremely low	Negligible
Very low	Very low	Very low	Very low	Extremely low	Extremely low	Negligible
Extremely low	Extremely low	Extremely low	Extremely low	Extremely low	Negligible	Negligible
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

2.5 Consequence assessment

The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring (OIE 2021a).

For this risk review, the steps taken to assess the 'likely consequences' associated with each hazard were:

- Identifying a likely outbreak scenario that may occur from host exposure to the hazard.
- Estimating the likelihood of that outbreak scenario occurring to obtain a 'partial likelihood of establishment and spread' for the outbreak scenario.
- Determining the level and magnitude of adverse impacts (economic, environmental and social) resulting from the outbreak scenario.
- Combining the 'partial likelihood of establishment and spread' with the corresponding estimation of impacts to obtain the 'likely consequences' for each exposure group.

2.5.1 Identification of the outbreak scenario

Several possible outbreak scenarios may follow exposure of a susceptible population to a hazard. These scenarios represent a continuum ranging from no spread, to establishment and spread of the disease to its natural geographic limits. For this risk review, the outbreak scenario assessed because it has the most potential to occur with significant consequences was that:

The hazard establishes in the directly exposed population and spreads to wild and farmed populations, is not eradicated, becomes endemic in Australia and eventually spreads to its natural geographical limits.

2.5.2 Partial likelihood of establishment and spread associated with the outbreak scenario

In determining the likelihood that the outbreak scenario would occur, consideration was given to the factors that influence the likelihood of the hazard establishing and spreading to other susceptible populations. The interaction between host, environmental and agent factors is critical to the likelihood of disease agent establishment or spread.

Several factors are relevant when estimating the partial likelihood of establishment and spread (PLES) for each hazard, including the:

- Likelihood that susceptible species would be exposed to an infectious dose.
  - The amount of each hazard present in the environment (through for example, shedding by infected animals), especially in the case of waterborne transmission, will affect the likelihood of establishment.
- Mechanisms of spread and transmission pathways.
  - Hazards that are directly transmitted and that do not require an intermediate host for completion of their life cycle are more likely to establish than those hazards with complex life cycles.
  - The ability of the hazard to survive in the environment outside a live fish.
- Presence and density of susceptible species.
  - The environmental conditions at the time of infection, the density of susceptible animals and the health and immunological status of the recipient host animal(s).
- Likelihood that infected animals would encounter susceptible species.
  - Animals infected with hazards which have a broad host range and that are widely distributed across Australia are more likely to make contact with a susceptible species than those animals infected with hazards with a limited host range and limited distribution.

Following review of the Ornamental fish IRA and the Gourami iridovirus review, this risk review made assumptions when estimating the PLES for each hazard, including:

- The likelihood of disease establishment and spread from an Australian ornamental fish population to other Australian ornamental fish populations depends on the type of facilities, their size, the range of fish species held and management practices.
  - If a disease were to occur in an Australian ornamental fish population being used to supply a significant part of the hobby sector or many breeders, then it could spread widely within this exposure group.
  - It is unlikely that hazards will spread from Australian ornamental fish to farmed foodfish due to the lack of exposure pathways and biosecurity practices expected to be in place in these facilities.

    - Use of marine ornamental fish as feed for farmed foodfish is unlikely to occur due to cost, required volume and nutritional requirements.

  - The likelihood of a hazard spreading from Australian ornamental fish populations to the wild via released fish will be affected by the reduced survivability of live marine ornamental fish in the wild due to their environmental sensitivity, especially to water temperatures.

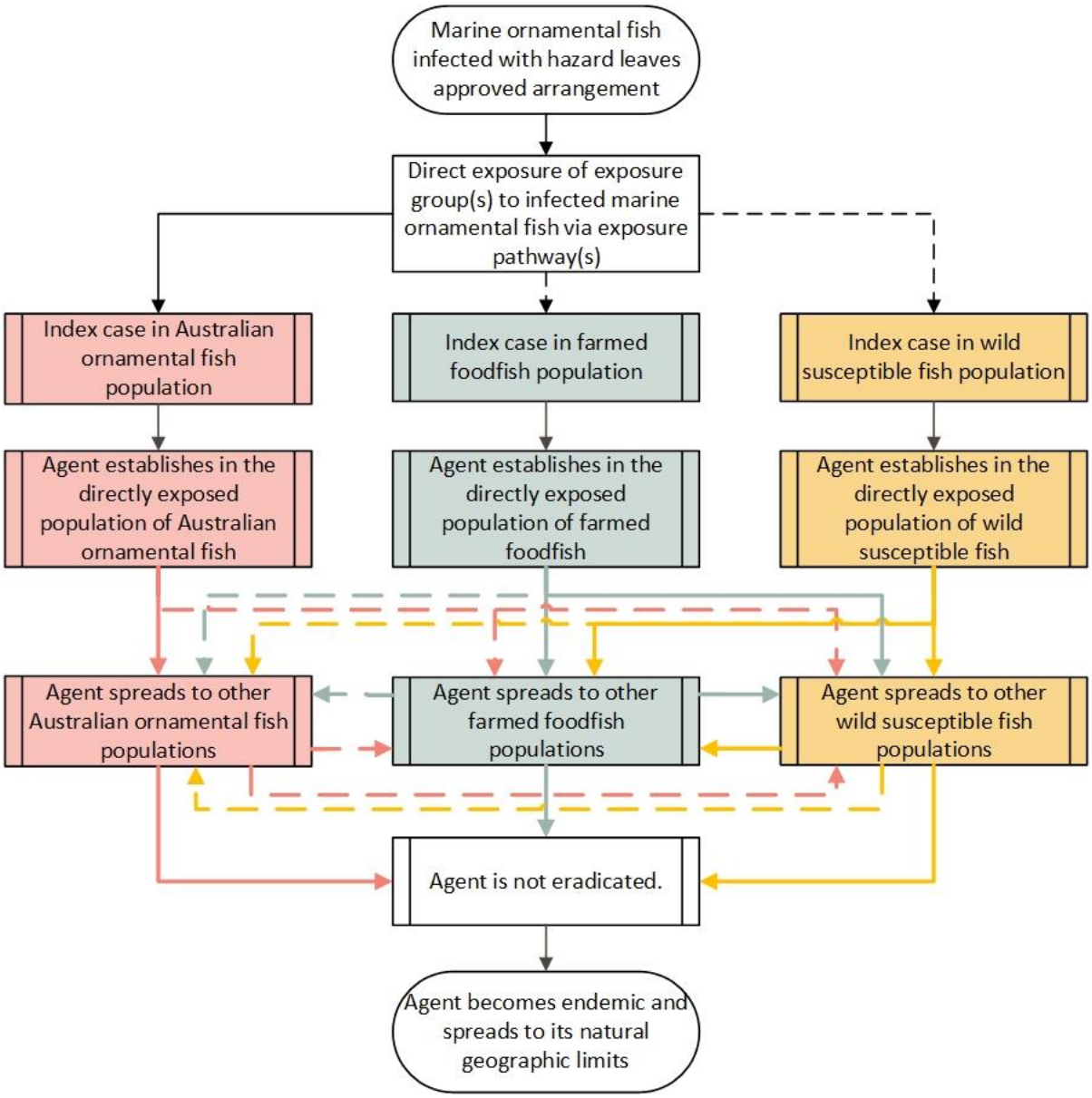
    - Hazard spread would be greater from breeding facilities compared to hobbyists as more fish are likely to be released *en masse* from facilities. This is because of the anticipated propagule pressure (the likelihood that there would be a successful establishment of a population from the release of an individual(s)) is strongly influenced by the number of individual animals released at the same time.

- The likelihood of disease establishment and spread from farmed foodfish to other exposure groups depends on the type of facilities, their size, the range of fish species held and management practices.
  - Spread from this exposure group to other farmed foodfish would depend on the species farmed, the type of facilities, management practices and the hazard host range.
  - Spread to the Australian ornamental fish population is unlikely given the closed systems and lack of exposure pathway between the two exposure groups.
  - Spread from farmed foodfish to the wild susceptible fish is the most likely scenario given the release of effluent water from foodfish farms into natural waters would be unlikely to inactivate any hazards present. Spread would be even more likely from cage operated farmed foodfish as they share water with wild susceptible species.
- If a hazard were to establish in the wild, the likelihood of spread to other exposure groups depends on the species affected and the operations of the facilities.
  - Spread and establishment within the wild will depend on the host range and characteristics of the hazard.
  - Spread from wild fish to Australian ornamental fish populations may occur in cases of subclinically affected species being collected from the wild for sale by the aquarium industry, but any clinically affected fish are unlikely to be harvested from the wild.
  - If disease were to establish in the wild, it may spread to cage operated farmed foodfish depending on their location and the species farmed. Spread to farmed foodfish in semi-closed or closed systems would depend on the biosecurity protocols in place and how much contact there is with environmental water.

Estimation of partial likelihood of establishment and spread

The likelihood of the outbreak scenario occurring for each exposure group is the PLES. The PLES for each exposure were estimated using the qualitative likelihood descriptors in Table 3 and is visually depicted in Figure 4.

Figure 4 Establishment and spread pathways for each exposure group



Establishment and spread pathways from the release of infected imported marine ornamental fish from the approved arrangement site through to the outbreak scenario being achieved and the agent becoming endemic and spreading to its natural geographic limits. Establishment and spread pathways depicted with full lines are more likely to occur than establishment and spread pathways depicted with dashed lines. Colour of lines denotes exposure group. Australian ornamental fish populations are pink boxes and lines. Farmed foodfish populations are green boxes and lines. Wild susceptible fish populations are yellow boxes and lines. White boxes and black lines apply to all exposure groups.

2.5.3 Adverse (economic, environmental and social) impacts

The potential adverse impacts of establishment and spread may be direct or indirect. They were evaluated against seven (two direct and five indirect) impact criteria.

Impacts may occur over an extended period and consideration of them is not limited to what might occur for one year but covers the period the impacts are discernible. The direct and indirect impacts described collectively cover the economic, environmental and social impacts of an outbreak—the so-called 'triple bottom line'. In assessing direct and indirect impacts, impacts were not considered

more than once and the frame of reference was the impact of each hazard on the Australian community, rather than on the directly affected parties.

Direct impacts
Direct impacts are those on the:

- Life or health (including production effects) of domestic or feral animals.
- Environment, including life and health of native wild animals and direct effects on the non-living environment.

Indirect impacts
Indirect impacts are those on:

- New or modified eradication, control, surveillance or monitoring and compensation strategies or programs.
- Domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries.
- International trade, including loss of markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand.
- Indirect effects on the natural environment, including biodiversity, endangered species, and the integrity of ecosystems.
- Indirect effects on communities, including reduced tourism, reduced rural and regional economic viability, loss of social amenity, and any 'side effects' of control measures.

The key considerations for the direct and indirect impacts as outlined in the Ornamental fish IRA and the Gourami iridovirus review, and as updated in this risk review, are summarised in Table 5.

Table 5 Summary of the key considerations for each of the direct and indirect impact criterion

Effects	Criteria	Key considerations
Direct	Animal health (production losses in aquaculture and commercial fisheries)	• The biological effect of disease is typically evaluated in terms of morbidity and mortality. • Diseases that reduce the efficiency of production without causing large increases in mortality are more likely to have a significant impact in Australian ornamental fish and farmed foodfish than wild susceptible fish. • Production losses will be impacted by the size of the facilities, range of fish species and management practices. • Only major epidemics involving significant mortalities or grossly visible clinical signs are likely to be detected in wild populations. • The consequences of establishment of an exotic disease in susceptible Australian populations is assessed in relation to characteristics of the local industry. • The burden of impacts of an outbreak of an exotic disease in Australia would be felt significantly more in the state(s) or territory(s) where the outbreak occurred, even when the impact is determined as being at a national level. • The value of the ornamental fish trade in Australia is currently unknown but it was last reported to be worth A$350 million annually, including both marine and freshwater species (Natural Resource Management Ministerial Council 2006). • The gross value of Australia's fish production in 2021–22 was estimated at A$1.97 billion from a total weight of 230,929 tonnes (Tuynman et al. 2023). The aquaculture industry produced 100,198 tonnes valued at A$1.40 billion while wild-caught fisheries produced 135,673 tonnes with an estimated value of A$594 million (Tuynman et al. 2023). • This risk review assumes that fish species in Australia would be at least as susceptible to infection as the same species, reported as susceptible under similar conditions in other countries.
	The environment (native animals/plants, and non-living environment)	• The establishment of a new disease could affect the survival of native species not farmed or otherwise commercially exploited. • To determine the likely effect of hazards on Australian native species, the department considered whether the hazards could infect a wide range of species, genera or families.
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	• Australia has a highly developed animal health system that can thoroughly investigate disease problems. • A high priority is placed, at both national and state and territory levels, on preventing exotic animal disease incursions. • Contingency planning for emergency aquatic animal diseases is well advanced at the national level. The department leads and coordinates the national management of aquatic animal health in Australia. • Australia's National Strategic Plan for Aquatic Animal Health (AQUAPLAN) is jointly developed by governments and private industry sectors. • Australia's Aquatic Veterinary Emergency Plan (AQUAVETPLAN) is a series of manuals that outline Australia's preparedness and response plans to deal with aquatic animal disease emergencies. • The costs associated with controlling and monitoring an aquatic animal disease outbreak would be substantial for the Australian, state and territory Governments and to the relevant farming and fishery industries (where relevant).

Effects	Criteria	Key considerations
		• A conservative approach was taken in this risk review, considering the high cost and time associated with attempts to eradicate new aquatic animal diseases and the challenges of success.
	Economic (domestic trade effects and impact on other associated industries)	• A disease outbreak may also have additional economic effects due to the loss of domestic markets, market oversupply and resulting reduction of prices received for product. Associated industries may also suffer significant production losses. • Indirect impacts would also likely affect aquaculture producers that are free of infection and would be most felt in those parts of Australia where fish farming (particularly those of susceptible species) makes a significant contribution to the overall local economy. • Public perception can significantly affect the markets for products intended for human consumption. • Domestic trade and movement restrictions may apply to wild susceptible species fished from areas impacted by an outbreak. Commercial wild catch industries would also be affected. Tourism and travel in affected marine environments may also be impacted. • It is not easy to quantify 'production' in the context of recreational fisheries. Although spending by recreational fishers is likely to provide economic and social benefits to rural and regional areas.
	Economic (international trade effects)	• Several countries have implemented strong import requirements for live, fresh and frozen fish species to prevent disease incursions. • The establishment of an exotic disease in Australia might have an adverse impact on export markets for Australian susceptible species. • In 2021–22, Australia exported 26,537 tonnes of salmonids (A$417 million) and 10,199 tonnes of tuna (A$135 million). An additional 10,459 tonnes of finfish, shark and ray products (from both aquaculture and wild fisheries sectors) valued at A$96.6 million were exported. • The major export destinations for Australian fisheries and aquaculture products were China, Hong Kong, Japan, United States of America and Vietnam (Tuynman et al. 2023). • In 2021–22, A$2.3 million of live ornamental fish were also exported (volume not available) (Tuynman et al. 2023). • If an exotic disease were to become established, Australia could use zoning to maintain access to international markets.
	Environment (biodiversity, endangered species and the integrity of ecosystems)	• The potential loss of biodiversity if a hazard were to be introduced, establish and spread, would be of concern to the Australian community. • A conservative approach was taken by the department when considering the susceptibility of native species, particularly those that are endangered or threatened, to infection with the hazards. • In drawing conclusions on the likely impact of exotic disease on the environment, the department considered overseas data on the susceptible species, the effect of infection and the influence of the physical environment on the outcome of infection.
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	• In the event of a disease outbreak, communities where aquaculture is a significant employer and/or plays a major role in the local community, are expected to experience the most significant social impacts.

Effects	Criteria	Key considerations
		• Loss of social amenity by recreational fishers because of the implementation of a movement regulated or restricted area could occur. • Loss of culturally significant species will also have social impacts if a disease outbreak were to affect important species for indigenous cultural fishing.

Determining impacts

Estimating the 'overall impact' associated with the outbreak scenario involved a two-step process where first, a qualitative descriptor of the impact of the hazard was assigned to each of the direct and indirect criteria in terms of the level of impact and the magnitude of impact. The second step involved combining the impacts for each of the seven criteria to obtain an 'overall impact' estimation.

Step 1: Assessing direct and indirect impacts

Each direct and indirect impact was estimated over four geographic levels, defined as:

- Local—an aggregate of households or enterprises (a rural community, a town or a local government area).
- District or region—a geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory, such as 'Far North Queensland').
- State or territory—a geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia).
- National—Australia wide (Australian mainland states and territories and Tasmania).

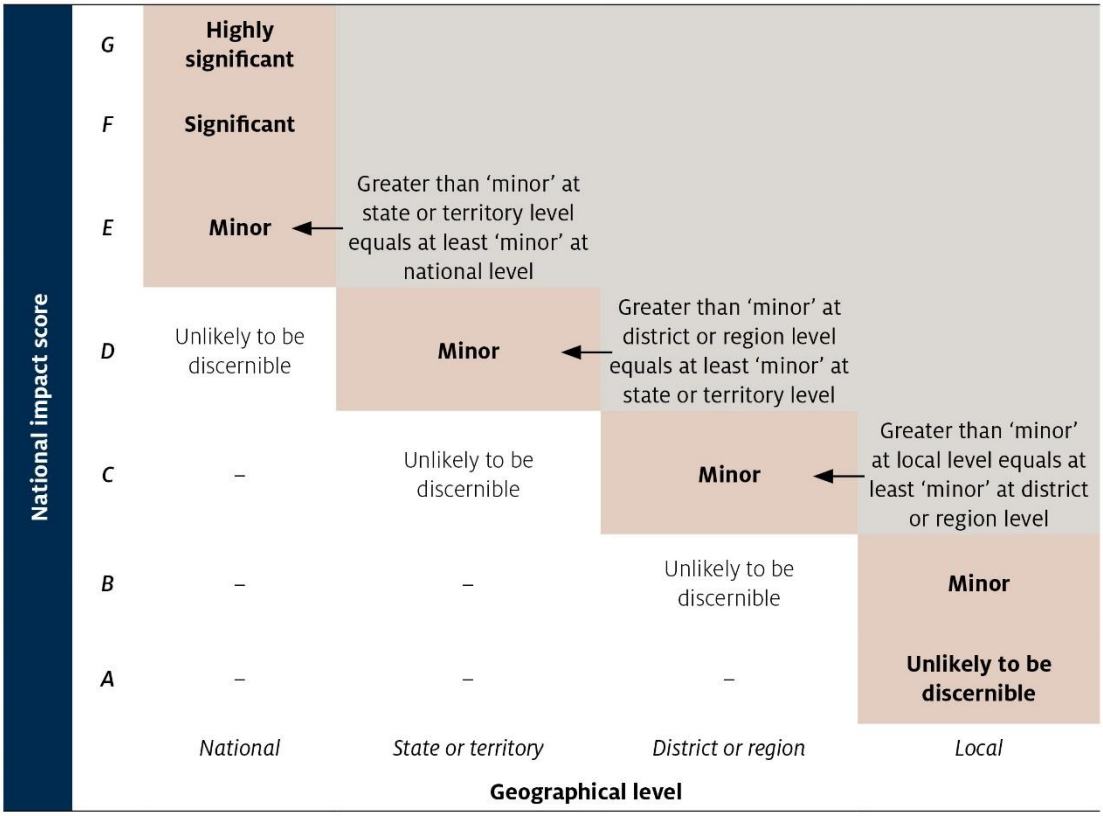
At each level, the magnitude of impact was described using four categories, defined as:

- Unlikely to be discernible—impact is not usually distinguishable from normal day-to-day variation in the criterion.
- Minor significance—impact is recognisable, but minor and reversible.
- Significant—impact is serious and substantive, but reversible and unlikely to disturb either economic viability or the intrinsic value of the criterion.
- Highly significant—impact is extremely serious and irreversible and likely to disturb either economic viability or the intrinsic value of the criterion.

Each individual direct or indirect impact was given an impact score (A–G) using the schema outlined in Figure 5. This was done by determining which of the shaded cells with bold font in Figure 5 corresponded to the level and magnitude of the particular impact. Additionally:

- At each geographic level below national, an impact more serious than 'minor' is considered at least minor at the level above. For example, a 'significant' impact at the state or territory level is considered equivalent to at least a 'minor' impact at national level.
- If the impact of a disease at a given level is in more than one state or territory, district or region or local area, it is considered to represent at least the same magnitude of impact at the next highest geographic level. For example, a 'minor' impact in multiple state or territories represents a 'minor' impact at national level.
- The geographic distribution of an impact does not determine the impact. For example, an outbreak could occur on one farm, but the impact could potentially still be considered at a state or national level.

Figure 5 Assessment of direct and indirect impacts on a national scale



Step 2: Combining direct and indirect impacts

The impact scores (A-G) for each direct and indirect criterion were combined to determine the 'overall impact' using the rules in Table 6. These rules are mutually exclusive and are assessed in numerical order until one applies. For example, if the first rule does not apply, the second rule is considered, and so on.

Table 6 Rules for combining direct and indirect impacts

Rule	Impact scores for each direct and indirect criteria	Overall impact
1	Any criterion has an impact of 'G'; or more than one criterion has an impact of 'F'; or a single criterion has an impact of 'F' and each remaining criterion an 'E'.	Extreme
2	A single criterion has an impact of 'F'; or all criteria have an impact of 'E'.	High
3	One or more criteria have an impact of 'E'; or all criteria have an impact of 'D'.	Moderate
4	One or more criteria have an impact of 'D'; or all criteria have an impact of 'C'.	Low
5	One or more criteria have an impact of 'C'; or all criteria have an impact of 'B'.	Very Low
6	One or more but not all criteria have an impact of 'B', and all remaining criteria have an impact of 'A'.	Negligible

2.5.4 Determination of likely consequences for outbreak scenario

'Likely consequences' for the outbreak scenario were determined by using the matrix in Figure 6 to combine the 'overall impact' (see Step 2: Combining direct and indirect impacts in section 2.5.3) with the 'likelihood of establishment and spread' (see section 2.5.2 Estimation of partial likelihood of establishment and spread).

When interpreting the matrix, note the vertical axis refers to *'likelihood of establishment and spread (PLES)'* and the horizontal axis refers to '*consequences of establishment and spread (impact score)'*. Accordingly, a '*low*' PLES combined with '*high*' impact, is not the same as a '*high*' PLES combined with '*low*' impact. This is because the matrix is not symmetrical.

Figure 6 Matrix for determining the 'likely consequences' for the outbreak scenario

Likelihood of establishment and spread (PLES)	Consequences of establishment and spread (impact score)					
	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
Very low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Extremely low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low

2.6 Estimation of overall annual risk

'Risk estimation' is the integration of 'likelihood of entry and exposure' and 'likely consequences' to derive the overall risk associated with entry, establishment and spread of a hazard.

Risk estimation was undertaken in two stages:

- Determining the partial annual risk (of entry, exposure, establishment and spread) for each of the three exposure groups.
- Combining the three partial annual risks to give an estimate of 'overall annual risk'.

2.6.1 Determination of partial annual risk

The partial annual risk (PAR) is the annual risk associated with each exposure group.

The PAR is determined by combining the PALEE (see section 2.4 Determination of the partial annual likelihood of entry and exposure) with the estimate of 'likely consequences' (see section 2.5.4 Determination of likely consequences for outbreak scenario) using the risk estimation matrix (Figure 7).

When interpreting the matrix, note the vertical axis refers to 'likelihood of entry and exposure (PALEE)' and the horizontal axis refers to 'consequences of entry and exposure ('likely consequences')'. Accordingly, a 'low' PALEE combined with 'high' likely consequence, is not the same as a 'high' PALEE combined with 'low' likely consequence. This is because the matrix is not symmetrical.

Figure 7 Matrix for determining the partial annual risk

Likelihood of entry and exposure (PALEE)	Consequences of entry and exposure ('likely consequences')					
	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
Very low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Extremely low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low

2.6.2 Estimation of overall annual risk

The overall annual risk is obtained by combining the PAR (see section 2.6.1 Determination of partial annual risk) for each of the exposure groups using the six rules outlined in Table 7.

These rules are mutually exclusive and are addressed in the order that they appear in the list. For example, if the first rule does not apply, the second rule is considered, and so on.

The result of this process was an estimate of the overall annual risk of introducing a hazard through importation of live marine ornamental fish. This is the final output of the unrestricted risk assessment.

Table 7 Rules for combining partial annual risks

Rule	Partial annual risks of the exposure groups	Overall annual risk rating
1	any one partial annual risk is extreme; or more than one partial annual risk is high; or any one partial annual risk high and each remaining partial annual risk is moderate.	Extreme
2	a single partial annual risk is high and the remaining partial annual risks are not unanimously moderate; or all partial annual risks are moderate.	High
3	one or more partial annual risks are moderate; or all partial annual risks are low.	Moderate
4	one or more partial annual risks are considered low; or all partial annual risks are very low.	Low
5	one or more partial annual risks are very low.	Very Low
6	all partial annual risks are negligible.	Negligible

2.7 Risk management

Australia has traditionally maintained a very conservative attitude to biosecurity risk. Given this, an overall annual risk that is either 'very low' or 'negligible', is sufficiently conservative to achieve Australia's ALOP. This provides a benchmark for evaluating risk and determining whether biosecurity measures are required.

2.7.1 Evaluating risk management options

The process for using a benchmark for evaluating risk is:

- For each hazard, the level of risk associated with the unrestricted importation of live marine ornamental fish was estimated using the process described in this chapter.
- The unrestricted risk was then evaluated to determine where it fell in relation to Australia's ALOP.
- If the unrestricted risk was 'negligible' or 'very low', then it was considered acceptable and further biosecurity measures were not required for that hazard.
- If the unrestricted risk was 'low', 'moderate', 'high' or 'extreme', then biosecurity measure(s) were identified (see chapter 3 Options for biosecurity management of imported live marine ornamental fish) and the risk was recalculated (referred to as 'restricted risk') with the biosecurity measure(s) applied.
- Where the subsequently restricted risk was 'very low' or 'negligible', that biosecurity measure(s) was considered acceptable for that hazard.

3 Options for biosecurity management of imported live marine ornamental fish

Biosecurity measures considered in this risk review are aimed at reducing the likelihood that the importation of live marine ornamental fish from any country would lead to the entry, exposure, establishment and spread of hazards in Australia. There are two means by which this may be achieved:

- Reducing the likelihood of hazards entering Australia in imported live marine ornamental fish.
- Reducing the likelihood that susceptible host animals in Australia would be exposed to the hazard in contaminated imported live marine ornamental fish or associated waste.

The biosecurity measures that could be applied to imported live ornamental fish to achieve Australia's appropriate level of protection (ALOP) were evaluated in the *Import risk analysis on live ornamental fish* released in 1999 (Ornamental fish IRA) (AQIS 1999) and partially considered in the *Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses—final import risk analysis report* (Gourami iridovirus review) (Department of Agriculture 2014). Whether those measures manage the risk associated with hazards in imported marine ornamental fish to achieve Australia's ALOP is reviewed here. Consideration of new biosecurity measures that could be applied in addition to Australia's current biosecurity measures for live marine ornamental fish are also provided in this chapter.

The biosecurity measures considered in this chapter were selected from a range of pre-export and on-arrival measures considered practicable and form the basis of the biosecurity measures that are recommended to apply to the importation of live marine ornamental fish. Alternative biosecurity measures that are demonstrated, to the satisfaction of Australian government authorities, to provide equivalent biosecurity would also be considered.

The Ornamental fish IRA concluded that several biosecurity measures would reduce the overall risk associated with the import of live marine ornamental fish to a level that achieves Australia's ALOP (see section 1.2.3 Existing policy). Those biosecurity measures are considered to still reduce risk to within Australia's ALOP, and they are considered further for each hazard during this risk review. Where it is determined that the current biosecurity measures on their own do not reduce the risk of a hazard to a level that achieves Australia's ALOP, the application of additional biosecurity measures, either in combination or singly, is considered.

3.1 Source population requirements

3.1.1 Sourcing from a country, compartment or zone free of a hazard

The importation of live marine ornamental fish could be permitted from countries, compartments or zones determined to be free of the hazard(s).

Determination of hazard freedom would need to be to a standard consistent with that recommended by the World Organisation for Animal Health (WOAH), or equivalent. To be satisfied that a country, compartment or zone is free of a given disease, the department must have formally recognised the competent authority of that country and be satisfied that the competent authority has the capacity for disease control, monitoring and surveillance as appropriate for the disease. It would be necessary for the disease to be subject to compulsory reporting or be the subject of consideration in disease investigation. The WOAH Aquatic animal health code (WOAH code) chapter 4.2 'Zoning and compartmentalisation' (WOAH 2023a), chapter 1.4, 'Aquatic animal health surveillance' (WOAH 2023a) and the relevant provisions in each disease chapter of the WOAH Code for 'self-declaration of country freedom', should be followed as a guide.

The Ornamental fish IRA did not recommend source freedom for any hazards in imported live marine ornamental fish when released in 1999 (AQIS 1999). However, *Carassius auratus* (goldfish) are required to be certified that they originate from a country, zone or export premises determined to be free from spring viraemia of carp virus (SVCV) and *Aeromonas salmonicida* (other than goldfish ulcer disease strains). This is to be done based on a) the absence of clinical, laboratory or epidemiological evidence of these disease agents in the source fish population in the previous two years; and b) a system of monitoring and surveillance for the previous two years acceptable to the Competent Authority and consistent with department's requirements.

The Gourami iridovirus review recommended additional biosecurity measures to manage the risk associated with megalocytiviruses in imported live gouramis (subfamilies Luciocephalinae and Macropodinae of the family Osphronemidae), bettas (*Betta splendens*), paradise fish (*Macropodus opercularis*), cichlids (family Cichlidae), and poeciliids (family Poeciliidae), including the option for sourcing the fish from a country, zone or compartment determined by the Competent Authority to be free from megalocytiviruses (Department of Agriculture 2023a) It was recommended that the competent authority determine country, zone or compartment freedom from megalocytiviruses consistent with the procedures described by *Additional health certification criteria and procedures* available on Australia's Biosecurity Import Conditions (BICON).

3.1.2 Removal of susceptible species from the permitted list

Some pathogenic agents of marine ornamental fish infect a wide range of species. For example, *Enteromyxum leei* affects many species of freshwater and marine fish. Other pathogenic agents have restricted host ranges, for example, similar damselfish ranavirus (SRDV) only affects *Pomacentrus similis* (similar damselfish). Restricting import or removing susceptible species from the List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) would reduce the likelihood of entry for those hazards where the host range is known. Application of this measure is most applicable when no other biosecurity measures are available. Where a whole genus is permitted import, measures would apply to all species in the genus unless evidence that not all species are susceptible to the hazard(s) was available.

Neither the Ornamental fish IRA nor the Gourami iridovirus review recommended removal of particular species from the *Permitted marine species list* because appropriate biosecurity measures were considered available to manage the biosecurity risk of the identified hazards.

3.2 Batch testing for hazards

Batch testing of imported marine ornamental fish may be recommended to reduce the entry likelihood of hazard(s) of concern. In general, the sampling regime should provide 95% confidence of detecting the hazard if it is present at a prevalence of 5% or greater. However, hazard specific parameters would be determined for any hazard requiring batch testing and would consider a range of hazard and host specific factors.

The level of protection provided by testing would depend amongst others, on the integrity of the sampling regime (including security of the batches), strict implementation of the sampling procedures (including appropriate random selection of samples), the availability of effective testing methods and the prevalence of the target agent in the batch of fish. Testing may be applied pre-border (pre-export) or on-arrival (at border). A combination of pre-export and on-arrival testing may also be used to improve the effectiveness of this biosecurity measure. In all cases, samples should be representative of the batch or source population of animals.

The Ornamental fish IRA did not recommend batch testing for any hazards in imported live ornamental fish when released in 1999 (AQIS 1999). However, the Gourami iridovirus review recommended additional biosecurity measures to manage the risk associated with *Megalocytivirus* in imported live gouramis, bettas, paradise fish, cichlids, and poeciliids, including the option for the batch of consigned fish to be tested by the Competent Authority and found negative for megalocytiviruses prior to export (Department of Agriculture 2014, 2023a). Batch testing requirements were recommended to be consistent with the procedures described by the department's *Additional health certification criteria and procedures* available on Australia's Biosecurity Import Conditions (BICON).

The Gourami iridovirus review also recommended that imported consignments of these fish species be subject to an on-going program of risk-based post-arrival verification testing for *Megalocytivirus* as a means of monitoring the effectiveness of overseas systems that underpin attestations about batch testing or source population disease freedom. The final Gourami iridovirus review report differed from the 2010 provisional final report in that industry concerns about the commercial feasibility of post-arrival batch testing were considered and as a result, recommended that batch testing occur prior to export. Many of these issues surrounding commercial feasibility are just as, if not more, relevant to marine ornamental fish. It is unlikely that on-arrival batch testing for marine ornamental fish could be practically implemented. This is because of the commercial and practical limitations associated with taking a statistically significant sample size for lethal testing from batches which contain small numbers of single species and high value individual animals. Should non-lethal testing and sampling methods (e.g. environmental DNA (eDNA) technology) be determined to be diagnostically suitable in the future, this could be considered as an equivalent biosecurity measure following consideration and assessment by the department.

For the purposes of demonstrating freedom from *Megalocytivirus*, the department recommends the appropriate test methods are based on PCR, and are capable of detecting subclinical carriers of the virus, such as two-step (nested) PCR and quantitative PCR methods published in peer-reviewed journal or equivalent.

For other hazards, testing methods should be at least to a standard consistent with the recommendations in the latest version of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH 2023g), or equivalent. To continue improving the effectiveness of biosecurity measures, the department may specify alternative methods with higher diagnostic sensitivity and/or specificity than the methods recommended by the WOAH, as new methods become available.

Systems outside those considered within this risk review would be considered on a case-by-case basis to determine if they are an equivalent biosecurity measure. As the effectiveness of testing for managing biosecurity risks may vary for different hazards, this option may be applied in combination with other measures to reduce the overall risk to an acceptable level.

3.3 Pre-export quarantine

Restricting imports to live marine ornamental fish that have undergone a pre-export quarantine (PEQ) period may reduce the likelihood of entry of some hazards. Holding marine ornamental fish bound for export to Australia in PEQ should restrict contact with other fish (and associated water and equipment) in the premises that may be infected with disease agents. PEQ will also enable the live marine ornamental fish to be monitored for a specified time to ensure they are free of visible signs of disease and parasites prior to export.

Current risk management measures for imported live marine ornamental fish include a pre-export quarantine period of 7 days. Increasing or decreasing quarantine periods may be considered pending the outcomes of risk assessment and based on the hazard specific technical information. However, specific information on incubation periods or carrier status of some hazards in marine ornamental is limited. Even with PEQ applied, subclinically infected marine ornamental fish or marine ornamental fish with low level parasite infestations may go undetected and could still be imported to Australia. This option was therefore not considered likely to reduce the entry likelihood of the hazards but is a recommended general biosecurity measure for marine ornamental fish.

3.4 Post-arrival quarantine

Subclinically infected fish carrying hazards of biosecurity concern may succumb to clinical infection during quarantine, if they are stressed by transport. Therefore, increasing the quarantine period may allow the expression of clinical signs of disease, indicating the presence of a particular hazard during biosecurity control. However, carrier fish that are transported and held under good conditions may carry hazards without showing obvious clinical signs and are likely to be released from quarantine irrespective of the length of the quarantine period. Also, increasing the quarantine period may not measurably reduce biosecurity risk for hazards which can be harboured for long periods without showing clinical signs of disease, as a biological relevant quarantine period may not be practical.

Current risk management measures for imported live marine ornamental fish include a post-arrival quarantine period of 7 days. Increasing or decreasing quarantine periods may be considered pending the outcomes of risk assessment and based on the hazard specific technical information. However, specific information on incubation periods or carrier status of some hazards is limited.

4 Hazard identification

For this review, the list of disease agents (potential hazards) of potential biosecurity concern was compiled from:

- Diseases listed by the World Organisation for Animal Health (WOAH) as affecting marine ornamental fish included on the department's List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A) (and other species where relevant) (Department of Agriculture 2023b; WOAH 2023b).
- Diseases identified in the Import risk analysis on live ornamental fish released in 1999 (Ornamental fish IRA) (AQIS 1999).
- Diseases identified in the Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses—final import risk analysis report (Gourami iridovirus review) (Department of Agriculture 2014).
- other diseases (including emerging) identified as occurring in marine ornamental fish.

Table 8 shows the list of potential hazards identified through this review and summarises the results of the hazard identification process, including the reason for removal or retention of each disease agent. The worldwide distribution of non-WOAH listed potential hazards is drawn from that reported in the literature. The worldwide distribution of WOAH listed potential hazards has been collected using WOAH-WAHIS (WOAH World Animal Health Information System) and other literature and information. Where an assessment of disease freedom is to be made by the department, it is based on evidence provided by the country of export.

All pathogenic agents of species included on the *Permitted marine species list* were considered potential hazards when compiling the list. However, a potential hazard could only be considered a hazard if it met the criteria outlined in section 1.6.1 Review of hazard identification.

The disease agents identified as hazards and retained for risk review are listed at the end of this chapter (see section 4.2 Disease agents retained for risk review).

4.1 Emerging diseases

The WOAH defines an emerging disease as:

> A disease, other than listed diseases, which has a significant impact on aquatic animal or public health resulting from a change of known pathogenic agent or its spread to a new geographic area or species; or a newly recognised or suspected pathogenic agent.

By its nature, the global trade in ornamental fish involves the movement of live animals outside of their original geographical range. This brings together new hosts and disease agents which may facilitate host-switching, and drive the emergence of new diseases and known diseases in new hosts (Feist et al. 2019; Peeler et al. 2011).

The department has ongoing media and scientific literature feeds about biosecurity issues for all animal species, those feeds are reviewed by technical experts. Information about emerging diseases

that are reported would likely be picked up through these information sources. Under the WOAH, Member Countries are required to notify of the occurrence of listed diseases and emerging disease events. Member Countries are also encouraged to provide the WOAH with other important aquatic animal health information.

Various information sources are used by the department to monitor emerging and existing disease agents of marine ornamental fish that may present a biosecurity risk to Australia. These include but are not limited to:

- aquatic animal disease experts
- departmental data on trade patterns
- grey literature (e.g. media reports)
- Network of Aquaculture Centres in Asia Pacific (NACA)
- WOAH
- other countries
- scientific conferences, webinars and workshops
- scientific literature.

A difficulty with emerging diseases is that there is often limited information available on which to assess the biosecurity risk. The department bases its decisions on biosecurity risk on the available science. Where evidence is lacking, a judgement is made based on the strength of the available information. This is done in a conservative way, assuming that the information available is accurate and represents the significance of the situation.

Information sources are constantly being reviewed by technical experts and if concerning information arises, the department will review the risk of the disease agent and determine if it achieves Australia's appropriate level of protection (ALOP). If the risk exceeds Australia's ALOP, then the department can take immediate action. It is important to note that these reviews are most often not a formal risk review, and therefore they are only released publicly if the review determines that changes need to be made.

Table 8 Hazard identification and refinement

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
Viruses								
Aquabirnavirus	A wide range of ornamental, foodfish, and other fish species, including: *Ctenolabrus rupestris* *Salmo salar* *Oncorhynchus mykiss*	No	Yes	Yes	Widely distributed	Yes	No: the aquabirnavirus strain used to experimentally infect *C. rupestris* is closely related to Tasmanian aquabirnaviruses, which is already present in Australia. The department will continue to monitor the situation with respect to aquabirnaviruses and marine ornamental fish.	(Crane et al. 2000; Davies et al. 2010; Gibson, Smail & Sommerville 1998; Mohr et al. 2015)
Aquareovirus	A wide range of ornamental, foodfish, and other fish species, including: *Pomacanthus semicirculatus* *Salmo salar* *Oncorhynchus* sp.	No	Yes	Yes	Widely distributed	No	No: the aquareovirus strain infecting *P. semicirculatus* is closely related to Tasmanian Atlantic salmon reovirus, which is already present in Australia. The department will continue to monitor the situation with respect to aquareoviruses and marine ornamental fish.	(Crane & Carlile 2008)
Damselfish neurofibromatosis virus (DNFV) (Neurofibromatosis)	Host range is limited to *Stegastes partitus*	No	Unknown	No	Bahamas Caribbean United States of America	No	No: DNFV host range is restricted to the bicolour damselfish *S. partitus*, which is not present in the wild in Australia. There is no evidence that native Australian fish species are susceptible to this virus.	(Campbell, Gibbs & Schmale 2001; Fishes of Australia 2015; Schmale, Gibbs & Campbell 2002; Schmale, Hensley & Udey 1983)

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
							DNFV is not included in Australia's National list of reportable diseases of aquatic animals. The department will continue to monitor the situation with respect to DNFV.	
Erythrocytic necrosis virus (ENV) (Viral erythrocytic necrosis, also known as piscine erythrocytic necrosis)	A wide range of ornamental, foodfish, and other fish species, including: *Rhinecanthus aculeatus* *Tautoga onitis* *Ecsenius midas* *Salmo salar* *Oncorhynchus mykiss* *Clupea pallasii* *Gadus morhua*	No	Yes	Yes	Widely distributed	Yes	No: present in Australia, and is not subject to control or eradication.	(Costa et al. 2023; Davies et al. 2009; Emmenegger et al. 2014; Nicholson & Reno 1981)
Herpesvirus causing fibropapillomatosis in sea turtles	Various sea turtles, and marine ornamental fish species: *Thalassoma duperrey*	No	Yes	No	No	No	No: infection with this virus causes benign tumours to develop on sea turtles. *T. duperrey* eats these tumours, resulting in positive infection, and for the species to be a potential vector of the virus to other sea turtles. Transmission between fish is not reported, nor are clinical signs in infected *T. duperrey.*	(Lu et al. 2000)

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
Megalocytivirus species Infectious spleen and kidney necrosis virus (ISKNV) and its three closely related genogroups: ISKNV genogroup, red sea bream iridovirus (RSIV) genogroup and turbot reddish body iridovirus (TRBIV) genogroup. (Infection with ISKNV, Red sea bream iridoviral disease (RSIVD), infection with TRBIV)	A wide range of ornamental, foodfish, and other fish species, including: *Pterapogon kauderni* *Platax orbicularis* *Lates calcarifer* *Oplegnathus fasciatus* *Pterophyllum scalare*	Yes (as Infection with RSIV)	Yes	No	Widely distributed across Asia	Yes (RSIV)	Yes: not present in Australia, is included on Australia's National list of reportable diseases of aquatic animals, has been detected in marine ornamental fish species, and can cause significant mortalities in susceptible fish.	(FRDC 2017; Go & Whittington 2006, 2019; Rimmer et al. 2016; United States Department of Agriculture 2022) (WOAH 2022c)
Nervous necrosis virus (NNV) strains (excluding Red-spotted grouper NNV) (viral nervous necrosis (VNN), viral encephalopathy and retinopathy (VER))	A wide range of ornamental, foodfish, and other fish species, including: *Ctenolabrus rupestris,* *Symphodus melops* *Labrus bergylta*	No	Yes	No	Widely distributed	No	Yes: not present in Australia, has been detected in marine ornamental fish species, and can cause significant mortalities in susceptible fish. VER is included on the Australia's National list of reportable diseases of aquatic animals.	(Korsnes et al. 2017)
Red-spotted grouper NNV (RGNNV)	A wide range of ornamental, foodfish and other fish species	No	No	Yes	Widely distributed	No	No: although VER is included on Australia's National list of reportable diseases of aquatic animals, RGNNV is widespread in Australia and is	(Moody et al. 2009)

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
							not subject to control or eradication.	
Similar damselfish ranavirus (SRDV) and related ranaviruses	Host range is currently limited to the ornamental and foodfish species: *Channa striata* *Lates calcarifer* *Pomacentrus similis*	Yes (as Infection with Ranavirus species)	Yes	No – other ranavirus species are present in Australia including Bohle iridovirus and epizootic haematopoietic necrosis virus	India	No (but Iridoviruses were considered as a group)	Yes: not present in Australia, has been detected in marine ornamental fish species, and can cause significant mortalities in susceptible fish.	(Sivasankar et al. 2017b)
Salmonid alphavirus (SAV) Also known as salmon pancreas disease virus and sleeping disease virus. (Salmon pancreas disease, pancreas disease, sleeping disease)	Primarily infects salmonids, but can infect species of marine fish including: *Labrus bergylta*	Yes	Yes	No	Distributed across Europe and the United Kingdom	No	Yes: not present in Australia, is included on Australia's National list of reportable diseases of aquatic animals, has been detected in marine ornamental fish species, and can cause significant mortalities in susceptible fish.	(Ruane et al. 2018; Snow et al. 2010; Wallace, McKay & Murray 2017)
Viral haemorrhagic septicaemia virus (VHSV)	Primarily a disease of salmonids but can infect other species of fish, including: *Centrolabrus exoletus* *Ctenolabrus rupestris* *Labrus* spp. *Symphodus melops*	Yes	Yes	No	Widely distributed	No	Yes: not present in Australia, is included on Australia's National list of reportable diseases of aquatic animals, and can cause significant mortalities in cultured susceptible fish.	(Ma et al. 2013; Munro et al. 2015)

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
	Macropharyngodon geoffroy							
Bacteria	-	-	-	-	-	-	-	-
Aeromonas salmonicida salmonicida Typical strain	Primarily a disease of salmonids but can infect fish species: *Labridae* spp.	No	Yes	No	Widely distributed	No	Yes: not present in Australia, is included on Australia's National list of reportable diseases of aquatic animals and can cause significant mortalities and economic loss.	(Treasurer & Laidler 1994)
Atypical *Aeromonas salmonicida*	Primarily a disease of salmonids but can infect fish species: *Labridae* spp.	No	Yes	Yes	Widely distributed	No	No: present in Australia, and is not subject to control or eradication.	(Treasurer & Laidler 1994)
Edwardsiella tarda	Wide range of fish, Including: *Holocentridae* spp. *Hippocampus erectus*	No	No	Yes	Widely distributed	No. It was considered but not retained for risk assessment.	No: present in Australia, and is not subject to control or eradication.	(Wang et al. 2020)
Francisella orientalis	A wide range of ornamental, foodfish, and other fish species, including: *Chromis viridis* *Cirrhilabrus* spp.	No	No	No	Widely distributed	No	No**:** there is not sufficient evidence that *F. orientalis* infects permitted species of marine ornamental fish. The department will continue to monitor the situation with respect to *F. orientalis* and marine ornamental fish.	(Camus et al. 2013)
Lactococcus garvieae (Streptococcosis of salmonids)	A wide range of ornamental, foodfish, and other fish species, including:	No	Yes	Yes	Widely distributed	No.	No: several strains are present in Australia, and is not subject to control or eradication.	(Choi et al. 2019; Eldar et al. 1999; Ortega et al. 2020)

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
	Pomacentrus moluccensis *Oncorhynchus mykiss*							
Mycobacterium species including: *Mycobacterium marinum* *Mycobacterium fortuitum*	Various ornamental and other fish species, including: *Chromis cyanea* *Abudefduf* spp.	No	Yes	Yes	Widely distributed	No	No: present in Australia, and is not subject to control or eradication.	(Giavenni & Finazzi 1980; Humphrey 1995)
Photobacterium damselae piscicida including: *Photobacterium damselae subsp. damselae*	Various aquatic animals, such as crustaceans, molluscs and marine fish: *Amphiprion ocellaris* *Pomacentrus caeruleus*	No	No	Yes	Widely distributed	Yes	No: present in Australia, and is not subject to control or eradication.	(Choi et al. 2019; Rivas, Lemos & Osorio 2013)
Pseudoalteromonas piscicida Also known as: *Flavobacterium piscicida* *Pseudomonas piscicida*	Various ornamental, foodfish, and other fish species, including: *Amphiprion clarkia* *Amblyglyphidodon curacao* *Salmo salar* *Oncorhynchus mykiss*	No	No	Yes	Widely distributed	No	No: present in Australia, and is not subject to control or eradication.	(Nelson & Ghiorse 2002)
Streptococcus iniae	Wide range of fish, Including: *Sparisoma aurofrenatum*	No	No	Yes	Widely distributed	No. It was considered but not retained for risk assessment.	No: present in Australia, and is not subject to control or eradication.	(Dhayanithi, Kumar & Kathiresan 2010; Keirstead et al. 2014)
Tenacibaculum spp., including:	A wide range of ornamental, foodfish, and other fish species, including:	No	No	Yes	Widely distributed	No	No: present in Australia, and is not subject to control or eradication.	(Avendaño-Herrara, Toranzo & Margariños 2006)

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
T. maritimum (tenacibaculosis)	*Rhinecanthus assasi* *Neoglyphieodon meles* *Pagrus major* *Salmonidae* spp. *Latris lineata*							(ICES 2019)
Photobacterium damselae piscicida including: *Photobacterium damselae subsp. damselae*	Various aquatic animals, such as crustaceans, molluscs and marine fish: *Amphiprion ocellaris* *Pomacentrus caeruleus*	No	No	Yes	Widely distributed	Yes	No: present in Australia, and is not subject to control or eradication.	(Choi et al. 2019; Rivas, Lemos & Osorio 2013)
Chlamydia, rickettsia								
Chlamydia spp. (Epitheliocystis)	Various crustacean and fish species, including: *Phycodurus eques*	No	Yes	Yes	Widely distributed	Yes	No: present in Australia, and is not subject to control or eradication.	(Meijer et al. 2006; Stride, Polkinghorne & Nowak 2014)
Rickettsia spp. excluding: *Piscirickettsia salmonis*	Various crustacean and fish species (both freshwater and marine), including: *Phycodurus eques*	No	No	Yes	Widely distributed	Yes	No: present in Australia, and is not subject to control or eradication.	(Davies 1986; Meijer et al. 2006)
Piscirickettsia salmonis (Piscirickettsiosis)	Primarily salmonids but has been detected by PCR in marine ornamental species, including: *Pinguipes chilensis* *Prolatilus jugularis*	Yes	Yes	No	Chile, Canada, Norway, Scotland and Ireland	Yes	No: there is not sufficient evidence that *P. salmonis* infects permitted species of marine ornamental fish. The department will continue to monitor the situation with	(Contreras-Lynch et al. 2015)

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
							respect to *P. salmonis* and marine ornamental fish.	
Fungi/oomycete								
Exophiala species	Various aquatic species, including fish species: *Phyllopteryx taeniolatus* *Gadus morhua*	No	No	Yes	Widely distributed.	No	No: present in Australia, and is not subject to control or eradication.	(Humphrey 1995; Landsberg et al. 1994; MAF Biosecurity New Zealand 2009; Nyaoke et al. 2009)
Fusarium solani	Various aquatic species, including fish species: *Pomacanthus* sp. *Holocanthus* sp. *Scarus* sp.	No	No	Yes	Widely distributed	No	No: present in Australia, and is not subject to control or eradication.	(Humphrey 1995; Neiffer et al. 2003; Stetter & Choromanski 1991)
Glugea spp.	Various aquatic species, including fish species: *Hippocampus erectus* *Vincentia conspersa*	No	Yes	Yes	Widely distributed	Yes	No: present in Australia, and is not subject to control or eradication.	(Bauer, Pugachev & Voronin 2002; Kayis 2011; Vagelli et al. 2005)
Ciliates								
Brooklynella hostilis (Clownfish disease)	Various ornamental, foodfish, and other fish species, including: *Amphiprioninae s*p. *Pomacanthus* sp. *Holacanthus* sp.	No	Yes	Yes	Widely distributed	Yes	No: present in Australia, and is not subject to control or eradication.	(Anshary, Sriwulan & Suriati 2020; Diamant 1998a; Landsberg 1995; Langdon 1990)
Protozoa								

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
Amyloodinium ocellatum	Various aquatic species, including fish species: *Amphiprion clarkii* *Dicentrarchus labrax*	No	No	Yes	Widely distributed	No	No: present in Australia, and is not subject to control or eradication.	(Humphrey 1995; Landsberg et al. 1994)
Cryptocaryon irritans (marine ich, marine "white spot" disease)	Various aquatic species, including fish species: *Amphiprion frenatus* *Pterois volitans*	No	No	Yes	Widely distributed	No	No: present in Australia, and is not subject to control or eradication.	(Diggles & Lester 1996; Erickson 2017)
Cryptosporidium species	Various aquatic species, including fish species: *Amphiprion percula* *Ctenochaetus* *Tominiensis* *Chromis viridis* *Chrysiptera hemicyanea*	No	Yes	Yes	Widely distributed	No	No: present in Australia, and is not subject to control or eradication.	(Golomazou & Karanis 2020; Zanguee et al. 2010)
Species in the order *Scuticociliatida*, such as: *Uronema marinum* *Miamiensis avidus*	Various aquatic species, including fish species: *Chromis viridis* *Heniochus acuminatus* *Apolemichthys trimaculatus*	No	No	Yes	Widely distributed	No	No: present in Australia, is and is not subject to control or eradication	(Humphrey 1995; Magalhães Cardoso et al. 2020; Power et al. 2019)
Metazoa								
Benedenia spp. including: *B. epinepheli* *B. melleni* *B. monticelli* *B. pargueraensis*	Various aquatic animals, including fish species such as: *Hemigymnus melapterus* *Dascyllus aruanus* *Lates calcarifer*	No	Yes	Yes	Widely distributed	Yes	No: the only species of *Benedenia* known to infect marine ornamentals are either already present in Australia, or have a lifecycle dependant on vector species	(Humphrey 1995; Lo & Morand 2001)

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Worldwide distribution	Hazard in 1999 ornamental fish IRA? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	Reference (s)
B. seriolae							that are not present in Australia.	
Cryptocotyle lingua	Various aquatic animals, including fish from the order Gobiiformes.	No	Yes	No	Europe, North America and Asia	Yes	No: The lifecycle of *C. lingua* is complex, and is dependent on vectors that are not present in Australia.	(Duflot et al. 2021; Kristoffersen 1991; Zander 2003)
Enteromyxum species excluding: *Enteromyxum leei*	A wide range of ornamental, foodfish, and other fish species, including: *Amphiprion* spp. *Chromis* spp. *Coris* spp. Blenniiformes	No	No	Yes	Widely distributed	No	No: some species are present in Australia, is not subject to control or eradication, and no significant adverse consequences are associated with the disease agent.	(Biosecurity New Zealand 2005; Noga 2000)
Enteromyxum leei	A wide range of ornamental, foodfish, and other fish species, including: *Amphiprion* spp. *Chromis* spp. *Coris* spp. Blenniiformes	No	Yes	No	Widely distributed	No	Yes: *E. leei* infects a wide host range, can transmit directly fish to fish, present as a subclinical infection, and cause economic consequence.	(Katharios et al. 2014; Katharios, Rigos & Divanach 2011; Özer et al. 2014; Yokoyama & Shirakashi 2007)

4.2 Disease agents retained for risk review

The disease agents identified as hazards and retained for risk review were:

- *Aeromonas salmonicida* subspecies *salmonicida*
- *Enteromyxum leei*
- Megalocytivirus limited to infectious spleen and kidney necrosis virus (ISKNV) and its genogroups: ISKNV genogroup, red sea bream iridovirus (RSIV) genogroup and turbot reddish body iridovirus (TRBIV) genogroup
- nervous necrosis virus
- Ranaviruses limited to similar damselfish virus and related viruses
- salmonid alphavirus
- viral haemorrhagic septicaemia virus.

5 *Aeromonas salmonicida* subspecies salmonicida

5.1 Background

Aeromonas salmonicida is one of the oldest known fish disease agents which causes acute to chronic disease syndromes of fish (Hiney & Oliver 1999). There are currently five subspecies of *A. salmonicida:*

- *A. salmonicida achromogenes*
- *A. salmonicida masoucida*
- *A. salmonicida pectinolytica*
- *A. salmonicida salmonicida*
- *A. salmonicida smithia* (Dallaire-Dufresne et al. 2014; Menanteau-Ledouble et al. 2016).

A. salmonicida salmonicida is the only strain described as 'typical' whereas all the other subspecies are described as 'atypical' (Wiklund & Dalsgaard 1998). *A. salmonicida salmonicida* is the only subspecies that complies with the criteria described in the World Organisation for Animal Health *Aquatic animal health code* (WOAH Code) Article 2.1.2 *Hazard Identification* (WOAH 2023a) and has been retained as a hazard.

A. salmonicida salmonicida, hereafter referred to as typical *A. salmonicida,* is the aetiological agent of furunculosis (Austin & Austin 2012). Furunculosis is a significant disease primarily affecting salmonids characterised by high morbidity and mortality (Dallaire-Dufresne et al. 2014). It is named after the furuncle or boil-like lesions that develop on the skin and musculature in chronically infected individuals (Dallaire-Dufresne et al. 2014). Susceptible host species include various freshwater and marine fish (Menanteau-Ledouble et al. 2016; Mohler 2003). Furunculosis was first reported by Emmerich and Weibel (1894) in Germany in a freshwater *Salmo trutta* (brown trout) hatchery and is now present in Africa, Asia, Europe, North and South America (Cipriano & Bullock 2001b; Hiney & Oliver 1999).

Infection with typical *A. salmonicida* is not listed as a disease notifiable to WOAH (OIE 2020) but it is on Australia's *National list of reportable diseases of aquatic animals* (AHC 2021). Australia has a long history of passive surveillance and a strong system in place to detect incursions. Typical *A. salmonicida* is considered exotic to Australia.

5.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of *A. salmonicida* is warranted.

5.2.1 Agent properties

Typical *A. salmonicida* is a non-motile, gram-negative rod bacterium classified within the family *Aeromonadaceae*, order *Aeromonadales* (Janda & Abbott 2010). Typical *A. salmonicida* has a genome that possess several mobile genetic elements and plasmids, which confer typical *A. salmonicida*

significant genetic diversity (Charette 2021). Mobile genetic elements and plasmid contain genes encoding potential virulence factors and genes for resistance to drugs (Charette 2021; Reith et al. 2008). Different strains of typical *A. salmonicida* have been identified and they vary in virulence (Dallaire-Dufresne et al. 2014). For example, bath exposure of *Oncorhynchus kisutch* (coho salmon) for 1 hour with 4 different strains of typical *A. salmonicida* resulted in mortalities of 100% for strain AS-1, 68% for AS-4, 36% for AS-3 and 0% for AS-5 (McCarthy 1983).

Typical *A. salmonicida* is often considered a cold water bacteria and a psychrophilic bacteria (Charette 2021). Psychrophilic means the bacteria can only grow at temperatures below 25°C or sometimes 30°C compared to mesophilic bacteria, which can also grow effectively at 37°C or above (Vincent & Charette 2022). Mesophilic bacterial strains have been identified within other *A. salmonicida* subspecies and there are even more that are still not attributed to any subspecies (Vincent & Charette 2022). There is a report of a mesophilic strain of *A. salmonicida* being isolated from a diseased warm water fish*, Epinephelus coioides* (orange-spotted grouper, estuary cod), with typical symptoms of furunculosis, which may suggest typical *A. salmonicida* includes both psychrophilic and mesophilic strains (Huang et al. 2020). The existence of psychrophilic and mesophilic strains of *A. salmonicida* is attributed to the genetic diversity within the species (Charette 2021).

Typical *A. salmonicida* can persist outside its host in marine, brackish and freshwater environments for an extended period (Hiney & Oliver 1999). It was reported by McCarthy et al (1977) to survive for 10 days in seawater (salinity 3.4%), 20 days in freshwater and 26 days in brackish water (salinity 2.34%) ((McCarthy 1977) cited in (Rose 1990)). Other studies showed survival in seawater was less than 10 days (Rose, Ellis & Munro 1990), was 17 days in river water (Allen-Austin, Austin & Colwell 1984) and 8 days in lake water (Morgan, Cranwell & Pickup 1991). Hiney (1994) reported survival in seawater and freshwater at 2–24 days and 2–63 days, respectively ((Hiney 1994) cited in (Department of Agriculture 2009)). Survival times in water are dependent on many factors including temperature, salinity, ultraviolet radiation and the presence of organic matter (Department of Agriculture 2009; Hiney et al. 2002; Rose, Ellis & Munro 1990).

Typical *A. salmonicida* can also persist and remain infectious in faecal and food waste sediment at the bottom of sea cages, freshwater tanks or in pond mud ((Hiney 1994) cited in (Department of Agriculture 2009)). McCarthy et al (1977) described extended survival (>29 days), but not multiplication, of typical *A. salmonicida* in unsterilised freshwater in the presence of detritus/sediment ((McCarthy 1977) cited in (Rose 1990)). In sterile water, typical *A. salmonicida* survived for longer periods in the presence of sediment (>21 days) compared to its absence (9 days) (Effendi & Austin 1994). It was also shown to remain viable for up to 276 days within a sediment–water mix (Hiney et al. 2002) and to retain its infectivity for 6–9 months in non-sterile pond mud ((Plumb 1999) cited in (Department of Agriculture 2009)).

Elevated temperature is considered a primary factor affecting the onset of clinical furunculosis for temperate fish species. Water temperatures of 15–20°C correlate with increased clinical signs of infection and with more rapid growth of typical *A. salmonicida* ((Malnar, Teskeredzic & Coz-Racovac 1988) cited in (Department of Agriculture 2009))(Lillehaug, Lunestad & Grave 2003; Sako & Hara 1981). However, furunculosis outbreaks can occur at temperatures as low as 2–4°C. Groberg et al (1978) showed that at 3.9°C and 6.7°C, mortality in fish experimentally infected by injection with

typical *A. salmonicida* varied from 2–26% among 3 *Salmonidae* species (*Oncorhynchus mykiss* (rainbow trout), *Oncorhynchus tshawytscha* (chinook salmon) and coho salmon) whereas at 20.5°C, 93–100% of these fish died within 2–3 days (Groberg et al. 1978).

Typical *A. salmonicida* can survive in dead fish stored at 4°C and when frozen for up to 50 days (Jakobsen et al. 2020)((Ferguson 1988) cited in (Department of Agriculture 2009)).

Typical *A. salmonicida* is sensitive to iodine (50 and 100mg/L), ethanol (50% and 70%), benzyl-4-chlorophenol/phenylphenol (1%), sodium hypochlorite (50, 100, 200, and 50,000 mg/L), n-alkyl dimethyl benzyl ammonium chloride (1:256), glutaraldehyde (2%) and potassium peroxymonosulfate-sodium chloride (1%) (Cipriano & Bullock 2001b; Mainous, Kuhn & Smith 2011).

5.2.2 Epidemiology

Host range

All *Salmonidae* species (salmonids) and *Anguillidae* species (eels) are believed to be susceptible to infection with typical *A. salmonicida* through natural exposure (Australian Government Department of Agriculture 2019; Dallaire-Dufresne et al. 2014). Non-salmonid species are also susceptible and it is proposed that few cultured or feral fish are immune (Cipriano & Bullock 2001a). Species which are reported to be susceptible to infection with typical *A. salmonicida* are summarised in Table 9.

Table 9 Genera susceptible to typical *Aeromonas salmonicida*

Environment	Family	Genus	Permitted import	Susceptible to infection with typical *A. salmonicida*
Marine	Labridae	All	Yes	N
	Pleuronectidae	*Hippoglossus*	No	E
	Serranidae	*Epinephelus*	No	N
Euryhaline	Salmonidae	All	n/a	N
	Acipenseridae	*Acipenser*	n/a	N
	Catostomidae	*Catostomus*	n/a	N
	Latidae	*Lates*	n/a	N, E
	Moronidae	*Dicentrarchus*	n/a	N, E
	Gadidae	*Gadus*	n/a	N
	Percidae	*Perca*	n/a	N
		Sander	n/a	E
	Petromyzontidae	*Petromyzon*	n/a	N
	Scophthalmidae	*Scophthalmus*	n/a	N, E
	Sparidae	*Sparus*	n/a	N
Freshwater	Anguillidae	All	n/a	N
	Cyprinidae	*Carassius*	n/a	N, E
	Gobionidae	*Coreius*	n/a	N
	Xenocyprididae	*Ctenopharyngodon*	n/a	E
	Leuciscidae	*Luxilus*	n/a	N
	Polyodontidae	*Polydon*	n/a	N, E

Permitted import Included on the department's List of permitted live marine ornamental fish suitable for import. **n/a** Not applicable, these species are not included on the department's List of permitted live marine ornamental fish suitable for import and are outside the scope of this review. **N** Susceptible to infection by natural exposure. **E** Susceptible to infection by experimental exposure.

Infection with typical *A. salmonicida* and the development of disease can occur in all life stages of fish (Boily, Malcolm & Johnson 2019; Coscelli et al. 2014b). There are differences in susceptibility of fish species to furunculosis. For example, rainbow trout are generally considered to be mostly resistant to infection with typical *A. salmonicida* whereas brown trout and *Salmo salar* (Atlantic salmon) are highly susceptible (Cipriano & Heartwell 1986; McCarthy 1983; Perez et al. 1996).

Geographical distribution

Typical *A. salmonicida* has been reported worldwide except for Australia and New Zealand (Hiney & Oliver 1999). It has been detected in Canada (Boily, Malcolm & Johnson 2019), Chile (Valdes et al. 2015), China (Yi et al. 2016), Denmark (EURL for Fish and Crustacean Diseases 2021), Germany (EURL for Fish and Crustacean Diseases 2021), Japan (Nomura, Kasai & Yoshimizu 2003), Ireland (EURL for Fish and Crustacean Diseases 2021), Italy (EURL for Fish and Crustacean Diseases 2021), Norway (Jarp et al. 1993), Spain (Toranzo & Barja 1992), Sweden (Wichardt, Johansson & Ljungberg 1989), Scotland (Treasurer & Laidler 1994) and United States of America (USA) (Cipriano et al. 2001; Ford, Cipriano & Penniston 1994).

Prevalence

Marine ornamental fish

Labridae (wrasse) species *Ctenolabrus rupestris* (goldsinny wrasse) and *Ctenolabrus exoletus* (rock cook wrasse) are the only confirmed instances of typical *A. salmonicida* infecting a marine ornamental species permitted for live import into Australia. The instance was limited to one outbreak event and no prevalence was estimated.

Salmon

Typical *A. salmonicida* has been found in farmed and wild salmonid species (Dallaire-Dufresne et al. 2014). For example, in Japan during 1979–2002, 22,109 propagated salmon were sampled for typical *A. salmonicida* that was detected at a prevalence of 12.2% in *Oncorhynchus keta* (chum salmon), 4.6% in *Oncorhynchus gorbuscha* (pink salmon) and 1.4% in *Oncorhynchus masou* (masu salmon) (Nomura, Kasai & Yoshimizu 2003). Typical *A. salmonicida* was detected at a prevalence of 14% (n=135) in fertilised eggs collected between 1995–2000 from a population of Atlantic salmon held at a facility in the USA (Cipriano et al. 2001). A survey of wild fish collected from Michigan rivers, USA in 2005–10 detected typical *A. salmonicida* at a prevalence of 9.6% (n=2115) with 20.5% (n=806) in chinook salmon, 6% (n=301) in Atlantic salmon, 2.8% (n=623) in coho salmon and 0.8% (n=385) in rainbow trout (Diamanka et al. 2013).There were 11 farm-level diagnoses of furunculosis in seawater-reared Atlantic salmon in British Columbia, Canada between 2002–16 (Boily, Malcolm & Johnson 2019). According to a report on fish diseases in Europe in 2020, there were at least 17 cases of typical *A. salmonicida* in trout (species not all specified) in Germany and 28 cases in various salmonids in Italy (EURL for Fish and Crustacean Diseases 2021). During 2013–17, 298 cases of typical *A. salmonicida* (confirmed or suspected) in 14 salmonid and 7 non-salmonid species were reported to the Canadian Food Inspection Agency (Boily, Malcolm & Johnson 2019).

Other fish

In 2004, 2.5% (n=118) of wild adult *Petromyzon marinus* (sea lamprey) collected from Lake Ontario, Canada tested positive for typical *A. salmonicida* (Faisal, Eissa & Elsayed 2007).

Mortalities

Marine ornamental fish

Known mortality events of permitted marine ornamental fish species are limited to wrasse species being used in aquaculture settings as cleaner fish. An outbreak of typical *A. salmonicida* in goldsinny wrasse and rock cook wrasse, housed with Atlantic salmon, in net pens in Scotland across 1991–92 caused a 55% mortality (n=58) (Treasurer & Laidler 1994).

No reports of infection, or of mortality events, in wild or aquarium wrasse caused by typical *A. salmonicida*, were found.

Other fish

Typical *A. salmonicida* causes a high rate of mortality in farmed salmonids, up to 100% in both natural infections and challenge trials (Boily, Malcolm & Johnson 2019; Cipriano et al. 2001; Dallaire-Dufresne et al. 2014).

Transmission

Typical *A. salmonicida* can be transmitted horizontally via water, contact between fish and contaminated equipment and food (Australian Government Department of Agriculture 2019; Cipriano & Bullock 2001b; Dallaire-Dufresne et al. 2014). Transmission from broodstock to progeny may be possible because typical *A. salmonicida* has been detected on the surface of fertilised eggs but is not thought to be a significant route of transmission (Cipriano & Bullock 2001b; Cipriano et al. 2001).

The bacteria is shed into the water by dead and live fish via faeces, urine and furuncular lesions (Enger et al. 1992; Hiney & Oliver 1999; Rose 1990). Shedding of typical *A. salmonicida* from dead or diseased Atlantic salmon and rainbow trout in freshwater and seawater ranged from 10^4–10^8 CFU/fish/hour (Perez et al. 1996; Rose, Ellis & Munro 1989). In freshwater where infected brown trout were held, 10^3–10^5 CFU/mL of typical *A. salmonicida* was detected ((Bullock & Stuckey 1977) cited in (Rose, Ellis & Munro 1989)*).* In an experimental study, the time between exposure of healthy chinook salmon to typical *A. salmonicida* (by cohabitation with infected fish) and bacterial shedding was 3 days (Ogut & Reno 2005). Typical *A. salmonicida* was isolated from tissues of dead fish up to 32 days post infection (dpi) and was present in the tank water for a further 8 days ((McCarthy 1977) cited in (Boily, Malcolm & Johnson 2019)).

Fish with subclinical infections or fish surviving disease outbreaks can act as carriers of typical *A. salmonicida* (Cipriano & Bullock 2001b; Perez et al. 1996). Carriers may continue to shed bacteria into the water column to infect the remaining population without themselves showing any clinical signs of infection (Hiney, Smith & Bernoth 1997). Rainbow trout survivors (mean weight 25 g) of experimental infection with typical *A. salmonicida* shed on average 3×10^4–10^5 CFU/fish/hour and the bacteria could still be recovered at 29 dpi from the freshwater (Perez et al. 1996). Stress associated with high stocking density, spawning, poor water quality, elevated water temperature or handling may cause carriers to progress to clinical disease (Australian Government Department of Agriculture 2019; Hiney & Oliver 1999).

Marine plankton, protozoa and other ectoparasites such as copepods (e.g. salmon lice), may act as vectors of typical *A. salmonicida* (Nese & Enger 1993). Bivalve molluscs can acquire typical *A. salmonicida* via filter feeding and then act as a temporary source of the bacteria and infect healthy fish (Starliper 2001). Effendi and Austin (1994) have similarly shown typical *A. salmonicida* is relatively short-lived in and on marine benthic invertebrates such as *Pagurus bernhardus* (common marine hermit crabs), *Homarus vulgaris (*European lobsters), *Peringia ulvae* (Laver spire shell) and *Marthasterias glacialis* (spiny starfish) as the bacterium could not be recovered after 2 days post exposure (Effendi & Austin 1994). Sediment is an important environmental reservoir of typical *A. salmonicida* as the bacteria can survive and retain its infectivity in faecal and food waste sediment at the bottom of sea cages, freshwater tanks or in pond mud ((Hiney 1994) cited in (Department of Agriculture 2009)).

Contaminated equipment has been implicated in the transmission of furunculosis (Wichardt, Johansson & Ljungberg 1989). For example, typical *A. salmonicida* can survive for up to 6 days on both wet and dry contaminated fish nets ((McCarthy 1977) cited in (Boily, Malcolm & Johnson 2019)). It was also demonstrated that typical *A. salmonicida* can adhere to solid surfaces, especially plastics and stainless steel such as found in aquaculture farm equipment that could act as a source of the bacteria (Carballo, Seoane & Nieto 2000).

In an experimental setting wrasse were taken from an aquaculture facility and were challenged by immersion with 1×10^5/mL (final concentration) of a virulent strain of typical *A. salmonicida*. Challenged wrasse failed to become infected, and displayed no clinical signs, nor experienced any mortalities (Treasurer & Laidler 1994). Wrasse species are unlikely to become infected with, or long-term carriers and transmitters of, typical *A. salmonicida* in typical farm settings (Hjeltnes et al. 1995). Instances where infection has occurred are considered exceptional, caused by long term exposure to, and consumption of, salmonids dead or dying of typical *A. salmonicida*.

Mechanism of spread

The mechanism of spread of typical *A. salmonicida* into new countries and/or areas has been attributed to the movement of fresh and marine fish species (Dallaire-Dufresne et al. 2014; EURL for Fish and Crustacean Diseases 2021).

Infectious dose

Although Atlantic salmon (mean weight 120 g) died when bath challenged with 1×10^5 cells/mL of typical *A. salmonicida*, there were no mortalities or bacteria isolated in challenged wrasse (Treasurer & Laidler 1994).

The minimum infective dose of typical *A. salmonicida* for Atlantic salmon (20–32 g) after short duration bath exposure (1–3 days) was 10^4 CFU/mL and after long duration exposure (3 weeks) was 10^2 CFU/mL (Rose, Ellis & Munro 1989). Intragastric intubation of Atlantic salmon (70–115 g) with a typical *A. salmonicida* dose of $>10^5$ CFU/fish was sufficient to establish infection (Rose, Ellis & Munro 1989). The minimum lethal dose (LD_{50}) of typical *A. salmonicida* for rainbow trout (25 g) following bath exposure for 12 hours was 10^8 CFU/mL and for turbot (30 g) was 10^5 CFU/mL (Perez et al. 1996). Intraperitoneal injection of 0.1 mL typical *A. salmonicida* suspensions at 10^4–10^7 CFU/mL resulted in a LD_{50} of 3×10^5 CFU/mL for rainbow trout (25 g) and 2×10^4 CFU/mL for turbot (30 g) (Perez et al. 1996). Injection of 50 CFUs of typical *A. salmonicida* or bath exposure in 10^7 CFU/mL for 45 minutes could induce mortality in Atlantic salmon (20 g) within 5–6 days (Nordmo & Ramstad 1997). A

concentration of $10^{4.8}$ CFU/mL typical *A. salmonicida* was required to cause 50% cumulative mortality in chinook salmon (1.2 g) by bath exposure (Ogut & Reno 2005). Atlantic salmon (approximately 600 g) injected with 100 µL of typical *A. salmonicida* (3.05×10^7 CFU/mL) induced mortality by 6 dpi (Yi et al. 2016). Cohabitation of healthy chinook salmon (mean weight 2 g) with infected donor fish (3 dpi following bath exposure for 24 hours to $10^{5.1}$ CFU/mL) resulted in 100% mortality over a 10-day period (Ogut & Reno 2005).

In a challenge study, injection of *Ctenopharyngodon idella* (grass carp) (mean weight 30 g) with $8 \times 10^5 – 8 \times 10^8$ CFU/mL typical *A. salmonicida* resulted in LD_{50} values of $1.28 \times 10^4 – 9.12 \times 10^5$ CFU/fish (Long et al. 2016). Bath exposure of turbot (11 g) to 10^6 CFU/mL typical *A. salmonicida* for 1 hour caused 100% mortality 7 dpi (Farto et al. 2011). The LD50 of typical *A. salmonicida* in *Carassius auratus* (goldfish) was 4.5×10^6 CFU/g following intraperitoneal injection (Lian et al. 2020). European seabass (mean weight 7.5 g), turbot (mean weight 5.0 g) and rainbow trout (mean weight 14.5 g) intraperitoneally injected with $2 \times 10^4 – 2 \times 10^7$ CFU/fish of typical *A. salmonicida* caused 100% mortality at all doses (Fernández-Álvarez et al. 2016). Intraperitoneal injection of *Hippoglossus hippoglossus* (Atlantic halibut) (weight 154–254 g) and Atlantic salmon (weight 93–289 g) with $10^3 – 10^8$ CFU/fish showed Atlantic halibut was more resistant to infection compared to Atlantic salmon, with 1.25×10^6 and $\leq 1 \times 10^2$ CFU/fish the minimum lethal doses, respectively (Bricknell et al. 1999).

5.2.3 Pathogenesis

Tissue tropism

Typical *A. salmonicida* gain entry to fish through the gills, mouth, anus and/or surface injury (Austin 1997; Farto et al. 2011). It has been found in the skin, muscle, mucus, gut, liver, kidneys, intestine, spleen, liver, heart and brain (Brocklebank 1998; Coscelli et al. 2014b; Farto et al. 2011; Hiney & Oliver 1999; Hiney, Kilmartin & Smith 1994).

Tissue titre

The titre of typical *A. salmonicida* detected on fertilised Atlantic salmon eggs ranged from $5.0 \times 10^2 – 1 \times 10^7$ CFU/g of egg (Cipriano et al. 2001). Dead chinook salmon from experimental infection had viable counts of typical *A. salmonicida* of $10^{7.2} – 10^{8.8}$ CFU/g of kidney tissue and $10^{2.8} – 10^{5.3}$ CFU/g of flesh (Stone, MacDiarmid & Pharo 1997). Carrier chum salmon and pink salmon have been found with $10^{3.7}$ CFU/g of kidney tissue and 10^6 CFU/mL of coelomic fluid ((Nomura, Yoshimizu & Kimura 1991, 1992) cited in (Stone, MacDiarmid & Pharo 1997)). Titres of 10^3 CFU/g were reported in the skin mucus of healthy brown trout (Hiney & Oliver 1999) and 10^6 CFU/g were detected in mucus of Atlantic salmon immediately prior to the onset of clinical disease (Cipriano et al. 1992). The titre of typical *A. salmonicida* present in the furuncle of experimentally infected Atlantic salmon was 10^{10} CFU/mL (Rose, Ellis & Munro 1989).

5.2.4 Diagnosis

Clinical signs

Marine ornamental fish

In wrasse, infection with typical *A. salmonicida* can result in no clinical signs or external signs such as lesions or bacterial microcolonies on muscles, gills, internal organs, or myocardial tissue (Kvenseth 1998; Treasurer 2012; Treasurer & Laidler 1994).

Other fish

In salmonids, infection with typical *A. salmonicida* can result in peracute (very severe disease of short duration often without apparent clinical signs), acute (rapid onset and/or a short course) or chronic (persistent or long-lasting) disease (Menanteau-Ledouble et al. 2016). Peracute infections are most common in fry and fingerlings and can cause the fish to become dark in appearance but often there are no clinical signs other than rapid death (Cipriano & Bullock 2001b; Dallaire-Dufresne et al. 2014; Hiney & Oliver 1999; Menanteau-Ledouble et al. 2016). Clinical signs of an acute infection can also include darkening of the fish and anorexia that are often noted 2–3 days before fish, typically smolts and juveniles, start to die in high numbers (Austin & Austin 2012; Dallaire-Dufresne et al. 2014; Hiney & Oliver 1999). Infected fish may also display lethargy, erratic swimming, respiratory distress, exophthalmia and external haemorrhagic lesions at the base of the fins and oral cavity (Cipriano & Bullock 2001b; Hiney & Oliver 1999). Furuncles involving skin, muscle or the viscera may be present but are usually restricted to the chronic infection (Cipriano & Bullock 2001b; Hiney & Oliver 1999). Chronic infection is typically reported in older fish (subadults and adults) that have become more refractive to the disease or in more resistant species (Brocklebank 1998; Cipriano & Bullock 2001b; Hiney & Oliver 1999). Chronically diseased fish are lethargic, anorexic and show darkening of the skin, exophthalmia, congested blood vessels at the base of fins, bloody discharge from the nares, experience low mortality and in many instances develop the characteristic furuncles (Cipriano & Bullock 2001b; Dallaire-Dufresne et al. 2014; Hiney & Oliver 1999). There can be evidence of healing of the furuncles in chronic infections (Department of Agriculture 2009). In some cases, such as when fish are subclinically infected or have survived an infection, no clinical signs are observed (Cipriano & Bullock 2001b; Hiney & Oliver 1999; Menanteau-Ledouble et al. 2016).

Infected eels, such as *Anguilla rostra* (American eel) and *Anguilla japonica* (Japanese eel), are characterised by discoloured patches on the skin and gills that can progress into deep ulcers that often involve the underlying muscle tissue. They also exhibit cranial swelling and corneal oedema (Noga & Berkhoff 1990). Turbot infected with typical *A. salmonicida* developed skin lesions on the head and operculum that similarly progressed into ulcers (Coscelli et al. 2014b). Other clinical signs included abnormal swimming and anorexia (Farto et al. 2011). Ulcerative lesions and haemorrhages have been described on the body surface of other infected species such as *Coreius guichenoti*, goldfish, European perch, and sea lamprey (Faisal, Eissa & Elsayed 2007; Lian et al. 2020; Long et al. 2016; Rupp et al. 2019).

Pathology

The pathological changes due to typical *A. salmonicida* are similar for a peracute and acute infection and include haemorrhage of the heart and viscera, softening of the kidney tissues, enlargement of the spleen and kidneys, pale liver with necrosis, necrosis on the gills and visceral congestion. During chronic infection, fish may exhibit haemorrhage in the muscles, liver, intestines, pyloric caeca and gills, enlarged spleen, kidney necrosis, visceral congestion, peritonitis and furuncles under the skin and in the muscle. The furuncles consist of necrotic tissue, tissue fluid exudate and macrophages (Austin & Austin 2012; Cipriano & Bullock 2001b; Dallaire-Dufresne et al. 2014; Hiney & Oliver 1999; Menanteau-Ledouble et al. 2016).

Histological examination of infected turbot showed the presence of dermal chronic granulomatous inflammation, haemorrhagic lesions in the kidney, liver and spleen, coelomitis, vascular congestion, perivascular oedema in kidneys and necrosis in the kidney, liver, spleen, pancreas, gills, thymus and

gonads (Coscelli et al. 2014a; Coscelli et al. 2014b; Farto et al. 2011). Splenomegaly was the only pathology observed in infected European seabass (Fernández-Álvarez et al. 2016). Infected goldfish displayed multifocal necrosis and infiltration of inflammatory cells in gill, liver, kidney and intestines (Lian et al. 2020).

Testing

Typical *A. salmonicida* can be cultured using standard bacteriological techniques and a combination of cellular and colonial morphology and biochemical characteristics can then be used to identify the species (Austin & Austin 2012; Cipriano & Bullock 2001b).

PCR methods are available for the detection and identification of typical *A. salmonicida* (Bartkova et al. 2017; Byers et al. 2002; Byers, Gudkovs & Crane 2002; Keeling et al. 2013). Immunological tests such as serum agglutination, immunoassays, immunofluorescence antibody test and enzyme linked immunosorbent assay can be used to confirm typical *A. salmonicida* infection (Hiney & Oliver 1999; Hiney, Kilmartin & Smith 1994; Sakai 1986; Saleh et al. 2011).

5.2.5 Treatment

Furunculosis can be treated by antibiotics such as oxytetracycline, fluroquinolone, florfenicol and sulfadimethoxine/ormetoprim (Boily, Malcolm & Johnson 2019; Noakes, Beamish & Kent 2000; Stoffregen et al. 1993). However, the development of antibiotic-resistant strains of typical *A. salmonicida* has rendered treatment therapies increasingly difficult and ineffective (Dallaire-Dufresne et al. 2014; McIntosh et al. 2008; Rose, Ellis & Munro 1989; Vincent et al. 2014).

5.2.6 Control and prevention

The salmonid farming industry has widely adopted a vaccine prepared from whole typical *A. salmonicida* bacterin, generally administered by intraperitoneal injection with an oil emulsion-based adjuvant, to manage furunculosis (Midtlyng 2014). However, the vaccine cannot be delivered to very small fish so they may become infected before vaccination (Hiney 1995; Midtlyng 2014). In addition, the vaccine has been linked to a variety of side effects including health problems, reduced fish production and reduced immune protection at low temperatures (Menanteau-Ledouble et al. 2016; Midtlyng 2014). The effectiveness of the classical bacterin vaccine in non-salmonid species is still unclear.

Improved management and husbandry practices have led to decreased mortality rates and outbreaks of clinical disease (Department of Agriculture 2009). For example, reducing stocking density has been shown to reduce mortalities during a furunculosis outbreak (Glenn & Taylor 2006). Stocking of farms with fertilised eggs or fish that have been certified to be free of typical *A. salmonicida*, treating incoming culture water by ultraviolet irradiation or ozonation, fallowing of aquaculture sites, and education of personnel can also reduce significant sources of potential contamination and prevent disease (Boily, Malcolm & Johnson 2019; Cipriano & Bullock 2001b; Skall & Olesen 2011). Chemical disinfectants such as Virkon (0.5–1% for 5 minutes), chlorine (200–500ppm for 10 minutes) and benzalkonium chloride (0.03% for 5 minutes) can be used to inactivate typical *A. salmonicida* on objects, hard surfaces and equipment (Bowker et al. 2016; Skall & Olesen 2011). Surface decontamination of eggs using iodine (50 mg/L for 30 minutes) is effective in preventing transmission of typical *A. salmonicida* from broodstock to progeny (Bowker et al. 2016; Cipriano & Bullock 2001b).

Selective breeding programs for fish strains that tolerate and/or resist infection with typical *A. salmonicida* have been in development since the 1970s with results showing an improvement in the resistance and commercial performance of selected family lines (Cipriano & Bullock 2001b; Cipriano & Heartwell 1986; Gjedrem 2010; Kjoglum et al. 2008; Zhang et al. 2011).

5.2.7 Impact of the disease

Prior to the introduction of an effective vaccine, furunculosis had a devastating impact on salmonid aquaculture resulting in large scale mortalities and economic losses (Dallaire-Dufresne et al. 2014). For example, the cumulative losses due to furunculosis in European salmon farming were estimated in excess of 20% per year ((Ellis 1997) cited in (Midtlyng 2014)). The development of injectable vaccines for typical *A. salmonicida* in the early 1990s reduced mortalities and increased production (Sommerset et al. 2005a). Furunculosis was still listed as a major concern for European rainbow trout farming in 2020 (EURL for Fish and Crustacean Diseases 2021). In 2019, Atlantic salmon production in Europe was 1.6 million tonnes and production of rainbow trout in seawater was 160,165 tonnes so an outbreak of furunculosis has the potential to cause significant financial losses (EURL for Fish and Crustacean Diseases 2021).

5.2.8 Current biosecurity measures

There are no biosecurity measures for typical *A. salmonicida* in marine ornamental fish.

There are biosecurity measures to manage the risk of typical *A. salmonicida* in goldfish imported to Australia for display purposes. These include that the goldfish must:

- originate from a country, zone or export premises (the population) determined to be free from *Aeromonas salmonicida* based on:
  - the absence of clinical, laboratory or epidemiological evidence of these agents in the source fish population in the previous 2 years, and
  - a system of monitoring and surveillance for the previous 2 years acceptable to the Competent Authority and consistent with the *Additional health certification criteria and for goldfish exported to Australia*
- have been inspected within 7 days prior to export and show no clinical signs of infectious disease
- have been in approved export premises for 14 days prior to export
- have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing)
- be inspected on arrival in Australia and moved directly to an approved arrangement site and remain for a minimum of 21 days.

More information can be found on the Australian Biosecurity Import Conditions (BICON) website.

5.2.9 Conclusion

Typical *A. salmonicida* is present in exporting countries, is not present in Australia and is capable of causing adverse effects. In Australia, infection with typical *A. salmonicida* is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

5.3 Risk assessment

Based on chapter 2 and the technical information about *A. salmonicida* presented in this chapter, the risk assessment was completed.

A summary of the risk assessment values are shown in Appendix D.

5.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for typical *A. salmonicida* were that:

- This risk analysis is generic and therefore the entry assessment assumes that typical *A. salmonicida* is present in all source countries.
- Reports of typical *A. salmonicida* infecting fish on the List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A), either naturally or experimentally, are limited to two species of wrasse (goldsinny wrasse, and rock cook wrasse).
- Instances of natural infection in a permitted species of marine ornamental are considered exceptional, with transmission caused orally through the high consumption of dead or dying infected salmonids (Treasurer & Laidler 1994).
- When challenged by immersion with a high viral load of typical *A. salmonicida*, wrasse did not show infection or mortality (Hjeltnes et al. 1995; Treasurer 2012; Treasurer & Laidler 1994).
- Wrasse have not been found to transmit typical *A. salmonicida* to other susceptible fish species. However, the bacterial load of typical *A. salmonicida* in any infected marine ornamental fish imported into Australia may be sufficient to cause infection in other susceptible species (Hjeltnes et al. 1995).
- Typical *A. salmonicida* can persist outside its host in marine, brackish and freshwater environments for an extended period.
- Pre-export inspection may detect marine ornamental fish showing clinical signs of infection with typical *A. salmonicida* and remove them before export. Marine ornamental fish sub-clinically infected or carrier fish would not be identified through visual inspection.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 3, the annual likelihood of entry of typical *A. salmonicida* was estimated to be **very low**.

5.3.2 Exposure assessment

The key points considered relevant when conducting the direct exposure assessment for typical *A. salmonicida* were that:

- Typical *A. salmonicida* can be transmitted via contact between fish and through water. Although only oral transmission has been observed in any permitted species of marine ornamental fish.
- Typical *A. salmonicida* can persist outside its host in marine, brackish and freshwater environments for an extended period.
- Typical *A. salmonicida* may be present in sufficient loads in imported fish to cause infection in susceptible species if exposed.

- Species susceptible to typical *A. salmonicida* infection are present in Australia including wrasse, salmonids, and genera *Epinephelus* (grouper, cod), *Gadus* (cod), *Perca* (perch) and *Sander* (pike perch).
- Water temperatures of 15–20°C correlate with rapid growth of typical *A. salmonicida* but outbreaks can also occur at temperatures as low as 2–4°C ((Malnar, Teskeredzic & Coz-Racovac 1988) cited in (Department of Agriculture 2009))(Lillehaug, Lunestad & Grave 2003; Sako & Hara 1981).
- Because of the holding conditions in aquarium facilities, any ornamental susceptible species grown with, or sharing the same water as infected imported fish, will likely be exposed to viable typical *A. salmonicida*. However, natural infection has only been detected in wrasse used as cleaner fish which are unlikely to be kept as ornamental fish.
- Farmed foodfish in Australia are known to be susceptible to typical *A. salmonicida* (e.g. salmonids). However, it is unlikely farmed fish would be in the vicinity of the shore where imported live marine ornamental fish would be released or that they could make their way to a fish cage. Feeding imported marine ornamental fish to farmed foodfish broodstock is assumed to be unlikely because of their high cost and the large volume and specific nutritional profile that farmed foodfish broodstock require. Further, there has been no evidence of infected wrasse transmitting typical *A. salmonicida*.
- Introduction into the wild may occur by direct release of imported marine ornamental fish or their associated waste/water into natural waters. This would be a direct pathway to wild susceptible species. However, the survival of marine ornamental fish released into the wild is expected to be reduced because of their environmental sensitivity.
- Typical *A. salmonicida* has a modest host range present in Australian waters that could be infected. However, wild susceptible species would likely be more scattered compared to the dense populations found in aquaculture, which would reduce opportunities for transmission.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the partial likelihood of exposure of each exposure group to typical *A. salmonicida* was estimated to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish—**Negligible**.
- Wild fish—**Extremely low**.

5.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to typical *A. salmonicida* was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish—**Negligible**.
- Wild fish—**Extremely low**.

5.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)

The key points considered relevant when determining the partial likelihood of establishment and spread for typical *A. salmonicida* were that:

- Typical *A. salmonicida* can be transmitted via contact between fish and through water.
- Typical *A. salmonicida* can persist outside its host in marine, brackish and freshwater environments for an extended period.
- It is likely that susceptible species in contact with typical *A. salmonicida*-infected fish would receive an infectious dose.
- Species susceptible to typical *A. salmonicida* infection are present in Australia including wrasse, salmonids, grouper and cod species.
- Typical *A. salmonicida* can be treated by antibiotics such as oxytetracycline, fluroquinolone, florfenicol and sulfadimethoxine/ormetoprim (Boily, Malcolm & Johnson 2019; Noakes, Beamish & Kent 2000; Stoffregen et al. 1993). Successful treatment reduces the likelihood of typical *A. salmonicida* establishing and spreading to its natural limits in an outbreak scenario.
- Infection with typical *A. salmonicida* in a permitted marine ornamental fish species has only been detected in wrasse utilised as cleaner fish, cohoused with foodfish. Whilst stressors associated with aquariums (e.g., stocking density, growing conditions) increases the likelihood of disease establishment, the lack of other known susceptible hosts, and ornamental fish not being cohoused with foodfish, makes it unlikely that typical *A. salmonicida* will establish in an Australian ornamental facility.
- Upon completion of on-arrival quarantine, fish could be transported through several ornamental facilities in Australia. Whilst it is unlikely typical *A. salmonicida* will establish in these facilities, there is an increased likelihood of spread if the disease is present in fish being translocated.
- Each state and territory have translocation protocols for aquaculture animals, which typically include consideration of typical *A. salmonicida,* which would reduce the likelihood of spread between farmed foodfish populations. Though the movement of ornamental fish between hobbyists and retail premises is difficult to monitor and not necessarily captured under all state and territory legislations.
- It is unlikely that typical *A. salmonicida* will spread from aquarium industry to farmed foodfish industry due to the lack of exposure pathways and biosecurity practices expected to be in place in facilities which farm susceptible species.
- It is unlikely that typical *A. salmonicida* will spread from an Australian ornamental facility to wild populations via released or escaped fish, given their environmental sensitivity and the anticipated propagule pressure required to ensure establishment of a population.
- Farmed foodfish in Australia are known to be susceptible to typical *A. salmonicida* (e.g. salmonids, grouper).
- If typical *A. salmonicida* were to establish in farmed foodfish populations it could spread to wild populations through release of water from farms into natural waters. Typical *A. salmonicida* can remain viable in water and sediment for months. The spread would be moderated by dilution effects and implementation of biosecurity measures. Spread from cage operated farmed foodfish to wild fish would be even more likely due to the sharing of water.

- Spread of typical *A. salmonicida* from foodfish facilities to the aquarium industry is unlikely given the closed systems and lack of exposure pathway between the two exposure groups.
- If one or more index cases of typical *A. salmonicida* were to occur in the wild, establishment and spread would be less likely than in a foodfish facility, given there is not the same density of hosts which reduces the opportunities for transmission. The ability of fish to be subclinically infected with typical *A. salmonicida* and to remain carriers after surviving an infection would aid its spread.
- If typical *A. salmonicida* were to establish in the wild, it may spread to cage operated farmed foodfish. If established in the wild, typical *A. salmonicida* may also spread to aquaculture facilities through water intake, due to horizontal transmission. The spread in saltwater and marine environments is unlikely to be moderated, due to typical *A. salmonicida* being able to survive in water and sediment for extended periods.
- In the absence of effective biosecurity measures, wild infected fish may be transferred into foodfish farms through the inlet water channels.

Conclusion
Based on these considerations and using the descriptors in Table 3, the partial likelihood of establishment and spread of typical *A. salmonicida* in each exposure group for the outbreak scenario was estimated to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish— **Moderate**.
- Wild fish—**Low**.

Determining adverse impacts resulting from the outbreak scenario
The factors considered relevant when determining the adverse impacts resulting from establishment and spread of typical *A. salmonicida* included:

Direct effects

The effect on the life or health (including production effects) of susceptible animals and fish

- Typical *A. salmonicida* causes high mortalities in farmed foodfish, including salmonids and grouper. Production and productivity losses due to typical *A. salmonicida* would be significant for the Australian salmonid industry with aquaculture production valued at approximately A$1.15 billion in 2021–22 (Tuynman et al. 2023). Grouper aquaculture industry is small in Australia, with only few research and commercial hatcheries (Rimmer et al. 1997).
- Infection with typical *A. salmonicida* has not been shown to cause high mortalities in susceptible species of permitted marine ornamental finfish. Losses due to typical *A. salmonicida* are unlikely to be significant for the marine ornamental industry.
- Typical *A. salmonicida* may impact wild fisheries in Australia. There are reports of typical *A. salmonicida* in wild fish overseas and associated mortalities although no reports of declines in catch rates.
- Based on the impacts of typical *A. salmonicida* infection overseas, typical *A. salmonicida* establishment and spread in Australia would be expected to cause significant impacts at the national level on the life or health of susceptible species.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There is a moderate host range for typical *A. salmonicida* in Australia.
- Infection with typical *A. salmonicida* has been reported to cause serious effects in wild fish populations overseas.
- The direct impact of typical *A. salmonicida* establishment and spread on the living environment is expected to be minor at the district or region level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with typical *A. salmonicida* is not listed as a notifiable disease by WOAH but is included on Australia's *National list of reportable diseases of aquatic animals*. State and territory governments would be expected to report on the presence of typical *A. salmonicida* in Australia.
- If typical *A. salmonicida* was confined to an ornamental or foodfish facility, then attempts at eradication may be undertaken, which is the preferred response strategy (Department of Agriculture 2009). The cost of eradication attempts in affected salmonid farms would be significant for the industry.
- To demonstrate that eradication is successful, there would need to be a surveillance exercise over at least two years to confirm freedom, at considerable cost.
- If a movement restricted area were put in place for typical *A. salmonicida*, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area. If typical *A. salmonicida* was confirmed in the environment, the inherent difficulties for the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating typical *A. salmonicida* from wild populations is unlikely to be undertaken.
- If eradication was unsuccessful, preventative vaccination programs may be implemented to control the spread of typical *A. salmonicida* or manage the production of a susceptible species if there was an economic benefit (Thorarinsson & Powell 2006). There is an effective commercial vaccine for typical *A. salmonicida* but its use in Australia would need to be approved and would increase the cost of aquaculture production (Midtlyng 2014).
- Eradication and control of typical *A. salmonicida* is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Movement control orders, if put in place, would have indirect impacts on other industries such as feed companies and aquaculture facilities due to the host range of typical *A. salmonicida*.
- Infected fish may show clinical signs which would affect their marketability.
- Typical *A. salmonicida* establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Whist infection with typical *A. salmonicida* is not WOAH-listed disease, importing countries may still have import requirements for live, fresh or frozen species susceptible to typical *A. salmonicida*.
- If typical *A. salmonicida* were to become established, Australia could use zoning to maintain access to international markets for live susceptible species.
- The impacts of typical *A. salmonicida* establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- Typical *A. salmonicida* has a moderate host range and has been reported in wild fish.
- No endangered Australian fish species are currently known to be susceptible to typical *A. salmonicida*.
- An *Epinephelus* species is susceptible to typical *A. salmonicida*. If typical *A. salmonicida* were to cause disease in *Epinephelus daemelii* (black rock cod), it could have an impact on the survival of this already vulnerable species.
- A conservative approach has been adopted in light of the susceptibility of native species.
- The impacts of typical *A. salmonicida* establishment and spread on environmental biodiversity is likely to be minor at the district or region level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Fish susceptible to typical *A. salmonicida* are recreationally fished in Australia and could be affected by mortalities and movement restriction areas put in place which may impact on social amenity. This includes impacts on important species for indigenous cultural fishing, such as perch, snapper and bream (DAFF 2003).
- In local areas where aquaculture is a major industry, a typical *A. salmonicida* outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of typical *A. salmonicida* establishment and spread are expected to be minor at the district or region level.

Table 10 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of typical *A. salmonicida*. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section Determining impacts for detailed methodology).

Table 10 Overall impact of establishment and spread of typical *A. salmonicida* for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Significant	F

Effects	Criteria	Level	Impact	Score
	The environment (native animals/plants, and non-living environment)	District or region	Minor	C
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	District or region	Minor	C
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	C

Conclusion

The overall impact of establishment and spread of typical *A. salmonicida* was estimated to be **high**.

5.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for typical *A. salmonicida* in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 6) and found to be:

- Australian ornamental fish—**Very low**.
- Farmed foodfish—**High**.
- Wild fish—**Moderate**.

5.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of typical *A. salmonicida* for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 7 and found to be:

- Australian ornamental fish—**Negligible**.
- Farmed foodfish—**Negligible**.
- Wild fish—**Negligible**.

5.3.7 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 7.

The overall annual risk associated with typical *A. salmonicida* was found to be **negligible**.

Therefore, as the overall annual risk achieves Australia's ALOP, specific biosecurity measures are not considered necessary for this hazard.

6 *Enteromyxum leei*

6.1 Background

Enteromyxum leei, formerly described as *Myxidium leei*, is the aetiological agent of enteromyxosis (Diamant 1992; Palenzuela, Redondo & Alvarez-Pellitero 2002). It is a highly pathogenic enteric parasite of marine and freshwater fish, including food and ornamental fish, and can cause heavy mortalities and significant financial losses within aquaculture industries (Padrós et al. 2001; Sitjà-Bobadilla & Palenzuela 2012). It is a member of the family *Enteromyxidae* in class Myxozoa (Palenzuela, Redondo & Alvarez-Pellitero 2002).

E. leei first emerged in the 1990s in farmed *Sparus aurata* (gilthead seabream) in the Mediterranean Sea and other cultured fish species (Diamant 1992; Diamant, Lom & Dyková 1994; Le Breton & Marques 1995; Tun et al. 2000). It has since been found to infect more than 60 marine species from 29 families, many of which are warmwater fish, across a wide geographical distribution (Sitjà-Bobadilla & Palenzuela 2012).

Infection with *E. leei* is not listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (WOAH 2023f) and is not on Australia's *National list of reportable diseases of aquatic animals* (AHC 2021). Australia has a long history of passive surveillance and a strong system in place to detect incursions. *E. leei* is considered exotic to Australia.

6.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of *E. leei* is warranted.

6.2.1 Agent properties

E. leei is a spore-forming obligate endoparasite that has a direct life cycle using fish as hosts (Diamant 1997). The bow-shaped spores range from 13.2−19 µm in length and 5−11 µm in width and have elongated polar capsules (6.2−9.8 µm length, 2.5−3.6 µm width) with 7 turns of the polar filament (Cuadrado et al. 2008; Diamant, Lom & Dyková 1994; Padrós et al. 2001). The infective stages for enteromyxosis are not the spores, but rather the developmental stages which are released from infected fish. *E. leei* has been classified in the family *Enteromyxidae*, in class Myxozoa (Palenzuela, Redondo & Alvarez-Pellitero 2002).

Myxozoan infections depend on water temperature. *E. leei* outbreaks have been reported at high water temperatures (Le Breton & Marques 1995; Yanagida et al. 2006). Several studies have established that *E. leei* actively proliferates in fish hosts kept at 20–25°C, but it cannot proliferate in fish kept in water lower than 15°C or higher than 26°C (Athanassopoulou, Prapas & Rodger 1999; China et al. 2013; Estensoro et al. 2010; Golomazou et al. 2014; Rigos et al. 1999; Yanagida et al. 2006; Yasuda et al. 2002). Although, it has been detected at 14.7°C in net caged *Diplodus puntazzo* (sharpsnout seabream) (Golomazou et al. 2014).

E. leei can remain viable for up to 24 hours in seawater at 20°C (Yokoyama et al. 2009). It is hypothesised that it may remain stable in seawater for longer if it is protected by parasite mucous

casts (Golomazou et al. 2006a; Yokoyama et al. 2009). Its viability and infectivity, however, are greatly reduced in hyposaline water (<9%) (Yokoyama & Shirakashi 2007).

6.2.2 Epidemiology

Host range

E. leei has a wide host range as summarised in Table 11 and presented in full in Appendix C.

Table 11 Genera susceptible to *Enteromyxum leei*

Environment	Family	Genus	Permitted import	*E. leei*	
				Susceptible to infection	Positive PCR; no active infection
Marine	Acanthuridae	*Acanthurus*	Yes	N	-
		Zebrasoma	Yes	N	-
	Batrachoididae	*Halobatrachus*	No	N	-
	Blenniidae	*Cirripectes*	No	N	-
		Parablennius	No	N	-
		Salaria	No	N	-
	Epinephelidae	*Epinephelus*	No	N	-
	Gobiidae	*Gobius*	No	N	-
	Labridae	*Cheilinus*	Yes	N	-
		Coris	Yes	N	-
		Labrus	Yes	N	-
		Symphodus	Yes	N	-
		Thalassoma	Yes	N, E	-
	Lutjanidae	*Lutjanus*	No	N	-
	Molidae	*Mola*	No	N	-
	Moronidae	*Dicentrarchus*	No	E	-
	Mugilidae	*Chelon*	No	N	-
	Mullidae	*Mulloidichthys*	Yes	N	-
		Mullus	Yes	N	-
	Nemipteridae	*Scolopsis*	No	N	-
	Oplegnathidae	*Oplegnathus*	No	N	-
	Paralichthyidae	*Paralichthys*	No	N	-
	Pleuronectidae	*Platichthys*	No	N	-
	Pomacentridae	*Amphiprion*	Yes	E	-
		Chromis	Yes	N	-
		Neopomacentrus	Yes	N	-
	Pomacanthidae	*Pomacanthus*	Yes	N	-
	Scaridae	*Sparisoma*	Yes	N	-
	Sciaenidae	*Sciaenops*	No	E	-
	Scophthalmidae	*Scophthalmus*	No	N	-

Environment	Family	Genus	Permitted import	*E. leei*	
				Susceptible to infection	Positive PCR; no active infection
	Scorpaenidae	*Scorpaena*	No	N	-
	Sparidae	*Diplodus*	No	N	-
		Pagellus	No	N	-
		Pagrus	No	N	-
		Sparus	No	N, E	-
		Spicara	No	N	-
	Tetraodontidae	*Takifugu*	No	E	-
Freshwater	Cichlidae	*Astronotus*	n/a	E	-
		Oreochromis	n/a	E	-
	Cyprinidae	*Puntius*	n/a	E	-
	Danionidae	*Danio*	n/a	E	-

Permitted import Included on the department's List of permitted live marine ornamental fish suitable for import. **n/a** Not applicable, these species are not included on the department's List of permitted live marine ornamental fish suitable for import and are outside the scope of this review. **N** Susceptible to infection by natural exposure. **E** Susceptible to infection by experimental exposure.

E. leei infects all life stages of fish (Sitjà-Bobadilla & Palenzuela 2012). Susceptibility varies with host species and age of fish (Padrós et al. 2001; Shin & Lee 2023; Sitjà-Bobadilla et al. 2007).

Geographical distribution

E. leei has a wide geographical distribution, including Mediterranean, Atlantic, Asian and South American regions. For example, it has been detected in Crete (Katharios et al. 2014), Cyprus (Diamant, Lom & Dyková 1994), France (Sakiti et al. 1996), Greece (Athanassopoulou, Prapas & Rodger 1999; Rigos et al. 1999), Japan (Tun et al. 2000; Yasuda et al. 2002), Israel (Diamant, Lom & Dyková 1994), Italy (Fioravanti et al. 2006), Republic of Korea (Sekiya et al. 2016), Republic of Türkiye (Özer et al. 2014), Spain (Branson, Riaza & Alvarez-Pellitero 1999; Padrós et al. 2001) and the United States of America (USA) (Hyatt et al. 2018).

Prevalence

Marine ornamental fish

In 2017, an outbreak of *E. leei* was detected in 4/16 different species of wild-caught, east African/Indo-Pacific tropical marine fish (n=228 fish) undergoing quarantine at a public aquarium within the USA (Hyatt et al. 2018).

Other fish

E. leei was detected at a prevalence of 80% (n=300) in sharpsnout seabream from 8 different fish farms from southern and central Greece between 1994–97 (Athanassopoulou, Prapas & Rodger 1999). Wild gilthead seabream individuals examined for *E. leei* during 2002–03 from the Israeli Red Sea had a prevalence of 7.8% (n=169) (Diamant, Colorni & Ucko 2004). From 2002–05, a parasitological survey was carried out on gilthead seabream from extensive and intensive farming systems in Italy and 13.8% (n=458) were positive for *E. leei* (Fioravanti et al. 2006). Prevalence of *E. leei* in 2 farms of *Pagrus major* (common sea bream) in Japan was 35% (n=20) in August 2006, but decreased to 0% in winter (Yanagida et al. 2008).

Mortalities

Marine ornamental fish

Wild-caught *Sparisoma cretense* (parrotfish) held in a public aquarium in Crete infected with *E. leei* experienced mortality exceeding 60% over a period of 3.5 months (Katharios et al. 2014).

Other fish

Diamant reported *E. leei* caused an outbreak in gilthead seabream cultured in southern Cyprus with mortalities at 5–10 fish/day in tanks of 5000 (Diamant 1992). The culture of sharpsnout seabream in Greece suffered significant (1–5% per day) and prolonged mortalities (3–6 weeks) due to *E. leei* with some epizootics resulting in 30–80% population loss (Athanassopoulou, Prapas & Rodger 1999; Rigos et al. 1999). Mortalities of 9% due to *E. leei* were reported in farmed common seabream in Japan in 2006 (Yanagida et al. 2008). In 2008, *E. leei* was also found in farmed *Epinephelus malabaricus* (Malabar grouper) in Japan and the cumulative mortality reached 20–50% among tanks (China et al. 2013).

Transmission

E. leei can be directly transmitted by cohabitation, cannibalism, ingestion, coprophagy and waterborne contamination (Alvarez-Pellitero, Palenzuela & Sitjà-Bobadilla 2008; Diamant 1997; Golomazou et al. 2006a; Sitjà-Bobadilla et al. 2007; Yanagida et al. 2008; Yasuda et al. 2002). The infective stages are shed from fish either via faeces or intestinal excretions (China et al. 2013; Diamant 1997). *E. leei* was detected at >10 cells/L in the culture water where clinical disease occurred whereas in the fish farms where clinical disease had not occurred it was detected at <10 cells/L (Sohn et al. 2021). Quantitative analysis of *E. leei* from rearing water of two *Paralichthys olivaceus* (olive flounder) farms reported a DNA copy number of 8×10^4 and 5×10^5 copies/L (Lee et al. 2021).

Water temperature is a critical risk factor in the transmission and onset of enteromyxosis (Sitjà-Bobadilla & Palenzuela 2012). Temperatures <15°C suppress *E. leei* transmission and proliferation, but both are returned with an increase in water temperature, indicating the capability of this parasite to become latent during cooler temperatures (Estensoro et al. 2010; Yanagida et al. 2006). As a result, fish can pass as false negatives in surveillance surveys during wintertime and become a source of the parasite to naive fish when the temperature rises (Estensoro et al. 2010).

Mechanism of spread

E. leei may be present subclinically in infected fish for months, making them hidden reservoirs of infection. These reservoirs, in combination with the ease of fish-to-fish transmission, contribute to the spread of the disease (Golomazou et al. 2014). Increased global connectivity and shorter transportation times also contribute to a higher probability and frequency that hosts and their parasites will be introduced to foreign locations and will arrive viable (Hallett, Hartigan & Atkinson 2015). In the case of ornamental fish, stress induced by transportation and entrance to a new environment likely play a role in inducing clinical disease and spread of the disease (Katharios, Rigos & Divanach 2011).

Infectious dose

There is no threshold level of parasite burden related to the emergence of mortality/morbidity (Fioravanti et al. 2020). In an experimental infection, *Amphiprion clarkia* (anemonefish), *Takifugu rubripes* (tiger puffer) and olive flounder fed intestine (ca. 0.1 g/1 g fish weight) from

infected olive flounder developed serious infection with *E. leei* and cumulative mortalities of 50%, 15% and 10%, respectively occurred (Yokoyama & Shirakashi 2007). *Amphiprion ocellaris* (clown anemonefish) exposed to a *E. leei* cell suspension of 1×10^5 parasites resulted in infection (Yokoyama & Shirakashi 2007). Olive flounder inoculated with 250 µL of *E. leei* intestinal scrapings (about 260 vegetative spores/µL) orally and anally resulted in infection (Shin et al. 2018).

6.2.3 Pathogenesis

Tissue tropism

The primary target organ for infection is the intestinal epithelium (Diamant 1997; Diamant, Lom & Dyková 1994; Fleurance et al. 2008; Tun et al. 2000). *E. leei* has also been detected in the gall bladder of some infected fish (China et al. 2013; Le Breton & Marques 1995; Özer et al. 2014; Rigos et al. 1999).

Tissue titre

There are limited publications about tissue titre of *E. leei* in wild or farmed fish. *E. leei* was reported in intensively sea caged sharpsnout seabream at a peak of 276 ± 63 copies/100 ng DNA in May for one cage (mean weight 37.5 g) and 781 ± 120 copies/100 ng DNA in June for a second cage (mean weight 11.2 g) (Golomazou et al. 2014). *E. leei* was detected at >50 cells/mg in olive flounder intestine samples from farms where enteromyxosis had occurred (Sohn et al. 2021). Another study on clinically-infected farmed olive flounder (weight 420 g) reported 1.3×10^8 copies/mg tissue in the intestine (Lee et al. 2021).

6.2.4 Diagnosis

Clinical signs

E. leei causes anorexia, anaemia, emaciation, cachexia, distended abdomen, scale loss and death (Athanassopoulou, Prapas & Rodger 1999; Diamant 1992, 1997; Padrós et al. 2001; Rigos et al. 1999; Tun et al. 2000; Yasuda et al. 2002). *E. leei* may also be present without showing clinical signs (Fleurance et al. 2008; Katharios et al. 2014; Padrós et al. 2001; Sitjà-Bobadilla et al. 2007).

Pathology

The pathology of *E. leei* is characterized by invasion of the intestinal mucosa, initially with little or no inflammatory response. In advanced, chronic infections, disruption of the integrity of the mucosa, desquamation and detachment of the epithelium occurs, and host tissue fragments, mucus and parasite stages may then be seen to accumulate in the intestinal lumen. Infiltration of mast cells and eosinophilic granular cells are also seen at the site of infection (Alvarez-Pellitero, Palenzuela & Sitjà-Bobadilla 2008; Athanassopoulou, Prapas & Rodger 1999; Diamant, Ram & Paperna 2006; Fleurance et al. 2008; Tun, Ogawa & Wakabayashi 2002; Yasuda et al. 2005). Enlarged or abnormally coloured gall bladders are common in some hosts (Sitjà-Bobadilla & Palenzuela 2012). Affected fish suffer osmoregulation failure and malabsorption of nutrients (Ishimatsu et al. 2007).

Testing

Microscopy and histology are historically used to detect *E. leei* (Athanassopoulou, Prapas & Rodger 1999; Diamant 1992; Diamant, Ram & Paperna 2006; Tun, Ogawa & Wakabayashi 2002; Yasuda et al. 2005). PCR (Palenzuela, Redondo & Alvarez-Pellitero 2002; Sekiya et al. 2016; Yanagida et al. 2005). qPCR methods are also available for diagnosis (Golomazou et al. 2014; Shin et al. 2018).

6.2.5 Treatment

There are currently no treatments for *E. leei* (Fioravanti et al. 2020).

Treatment of infected fish with a combination of amprolium and salinomycin have been shown to be partially effective in reducing prevalence and mortalities in some trials (Golomazou et al. 2006b; Hyatt et al. 2018). Water temperatures above 25−30°C or below 15°C have also been shown to reduce mortalities and clear the infection by inhibiting *E. leei* development (Athanassopoulou, Prapas & Rodger 1999; China et al. 2013; Estensoro et al. 2010; Golomazou et al. 2014; Rigos et al. 1999; Yanagida et al. 2006; Yasuda et al. 2002). Feed supplemented with commercially available health-promoting additives were experimentally shown to reduce infection level and severity (Palenzuela et al. 2020).

Susceptible hosts can develop adaptive immunity after natural recovery from infection with *E. leei* (Estensoro et al. 2010; Fleurance et al. 2008; Picard-Sánchez et al. 2019; Sitjà-Bobadilla et al. 2007; Sitja-Bobadilla et al. 2015). For example, it has been shown that gilthead sea bream that had survived infection with *E. leei* maintained high levels of specific anti- *E. leei* IgM (up to 16 months), expressed high levels of immunoglobulins at the intestinal mucosa, and were resistant to re-infection (Picard-Sánchez et al. 2019).

6.2.6 Control

Prevention is the main focus for the management of *E. leei* such as testing source stocks for parasites, quarantining new stocks, performing parasitological checks, feeding fish adequate diets, maintaining low fish densities, removing deceased fish frequently, avoiding recirculation systems and year-round elevated water temperatures, ensuring good water exchange, treating incoming water (ozone, UV and filtration), disinfecting effluent water and cleaning tanks, pipes and cages with freshwater or disinfectants. This is because once parasites become established, they are generally eradicated only with aggressive actions that include eliminating infected fish, disinfecting tanks and sea cages and drying ponds (Fioravanti et al. 2020; Hallett, Hartigan & Atkinson 2015; Jublanc et al. 2005; Sitjà-Bobadilla & Palenzuela 2012).

6.2.7 Impact of the disease

E. leei outbreaks have had an enormous impact on aquaculture and aquarium industries worldwide (Fioravanti et al. 2020; Padrós et al. 2001; Rigos et al. 1999; Rigos & Katharios 2010). Infections in gilthead seabream farms in the Mediterranean sea and Atlantic ocean sites have had serious economic consequences, and it has caused the stagnation or abandonment of wide-scale production of valuable fish such as sharpsnout seabream, tiger puffer or *Pagrus pagrus* (red porgy) (Fioravanti et al. 2020). In the Republic of Korea, the production of olive flounder decreased from 26,093 tonnes in 2016 to 21,463 tonnes in 2021 with the mortality caused by *E. leei* infections a major contributing factor (Shin & Lee 2023). The impact of *E. leei* is not limited to direct mortality but also to weight loss, poor conversion efficiency, delayed growth, secondary infections and reduced marketability (Golomazou, Karagouni & Athanassopoulou 2004; Katharios et al. 2014; Özer et al. 2014; Sitjà-Bobadilla & Palenzuela 2012).

6.2.8 Current biosecurity measures

E. leei was not assessed in the 1999 *Import risk analysis on live ornamental fish* (Ornamental fish IRA) (AQIS 1999) and there are no current biosecurity measures specific for *E. leei*.

6.2.9 Conclusion

E. leei is present in exporting countries, is not present in Australia and is capable of causing adverse effects. In Australia, infection with *E. leei* is not a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

6.3 Risk assessment

Based on chapter 2 and the technical information about *E. leei* presented in this chapter, the risk assessment was completed.

A summary of the risk assessment values are shown in Appendix D.

6.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for *E. leei* were that:

- This risk review is generic and therefore the entry assessment assumes that *E. leei* is present in all source countries.
- There are reports of *E. leei* infecting marine ornamental fish on the List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A). This includes species from the families Labridae (wrasse), Pomacentridae (damselfish and clownfish), Acanthuridae (surgeonfish, tang, and unicornfish), Mullidae (goatfish) Pomacanthidae (marine angelfish), and Scaridae (Parrotfish).
- *E. leei* has been detected in wild-caught marine ornamental fish but the prevalence was not reported. Prevalence in farmed foodfish can reach up to 80%.
- *E. leei* can survive in seawater less than 24 hours (Yokoyama et al. 2009).
- The parasite load of *E. leei* in infected imported fish would likely be sufficient to cause infection in susceptible species.
- Pre-export inspection may detect ornamental fish showing clinical signs of infection with *E. leei* and remove them before export. Fish sub-clinically infected would not be identified through visual inspection.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 3, the annual likelihood of entry of *E. leei* was estimated to be **high**.

6.3.2 Exposure assessment

The key points considered relevant when conducting the direct exposure assessment for *E. leei* were that:

- *E. leei* can be transmitted via contact between fish and through water.
- *E. leei* can survive for less than a day in seawater.
- *E. leei* would be expected to be present in sufficient loads in imported fish to cause infection in susceptible species if exposed.
- Species susceptible to *E. leei* infection are present in Australia include wrasse, damselfish, clown fish, goatfish, marine angelfish and genera *Cirripectes* (blennies), *Epinephelus* (groupers, cod), *Lutjanus* (common snappers), *Mola* (sunfish), *Oplegnathus* (knifejaw fish), *Pagrus* (seabreams),

Parablennius (combtooth blennies), *Paralichthys* (large-tooth flounders), *Scolopsis* (threadfin breams), *Scorpaena* (scorpionfish) and *Sparus* (gilt-head seabreams).

- *E. leei* infects fish at water temperatures 15–25°C (Estensoro et al. 2010; Yanagida et al. 2006; Yokoyama et al. 2009).
- Because of the holding conditions in aquarium facilities, any ornamental susceptible species grown with, or sharing the same water as infected imported fish, will likely be exposed to viable *E. leei*.
- There have been 3 reports of *E. leei* outbreaks in marine fish in ornamental aquariums overseas affecting wild captured fish from different species, in which the infection was spread to the majority of fish sharing the same water in the aquarium (Hyatt et al. 2018; Katharios, Rigos & Divanach 2011; Padrós et al. 2001).
- Farmed foodfish in Australia are not known to be susceptible to *E. leei* (e.g. salmonids, tuna, barramundi).
- Introduction into the wild may occur by direct release of imported marine ornamental fish or their associated waste/water into natural waters. This would be a direct pathway to wild susceptible species. However, the survival of marine ornamental fish released into the wild is expected to be reduced because of their environmental sensitivity.
- *E. leei* has a modest host range present in Australian waters that could be infected. However, wild susceptible species would likely be more scattered compared to the dense populations found in aquaculture, which would reduce opportunities for transmission.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the partial likelihood of exposure of each exposure group to *E. leei* was estimated to be:

- Australian ornamental fish—**Moderate**.
- Farmed foodfish—**Extremely low**.
- Wild fish—**Very low**.

6.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to *E. leei* was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Australian ornamental fish—**Moderate**.
- Farmed foodfish—**Extremely low**.
- Wild fish—**Very low**.

6.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)
The key points considered relevant when determining the partial likelihood of establishment and spread for *E. leei* were that:

- *E. leei* can be transmitted via contact between fish and through water.
- *E. leei* is estimated to survive for only one day in seawater.

- It is expected that susceptible species in contact with *E. leei*-infected fish would receive an infectious dose.
- Species susceptible to *E. leei* infection are present in Australia include wrasse, damselfish, clown fish, goatfish, marine angelfish, blennies, groupers, common snappers, sunfish, knifejaw fish, seabreams, combtooth blennies, large-tooth flounders, threadfin breams, scorpionfish, gilt-head seabreams.
- *E. leei* infects fish at water temperatures 15–25°C (Estensoro et al. 2010; Yanagida et al. 2006; Yokoyama et al. 2009).
- There are no commercially available treatments for *E. leei* infections.
- *E. leei* could establish in ornamental susceptible species. This is due to the stressors associated with aquariums. For example, the higher density of susceptible animals and the holding conditions.
- Fish could be moved to other ornamental facilities in Australia. It is expected that *E. leei* would establish in these facilities if present in the fish being translocated.
- Each state and territory have translocation protocols for aquaculture animals, which may not include consideration of *E. leei*.
- If *E. leei* were to establish in ornamental facilities supplying a significant part of the hobby sector or many breeders, it could spread widely within this exposure group.
- It is unlikely that *E. leei* will spread from aquarium industry to farmed foodfish industry due to the lack of exposure pathways and biosecurity practices expected to be in place in facilities which farm susceptible species.
- It is unlikely that *E. leei* will spread from ornamental facility to wild populations via escaped fish, given their environmental sensitivity and the anticipated propagule pressure required to ensure establishment of a population.
- Farmed foodfish in Australia are not known to be susceptible to *E. leei* (e.g. salmonids, tuna, barramundi).
- If *E. leei* were to establish in farmed foodfish populations it could spread to wild populations through release of water from farms into natural waters, but this would be limited due to its survivability of <24 hours in seawater. The spread would also be moderated by dilution effects and implementation of biosecurity measures. Spread from cage operated farmed foodfish to wild fish would be more likely due to the sharing of water.
- Spread of *E. leei* from foodfish facilities to the aquarium industry is unlikely given the closed systems and lack of exposure pathways between the two exposure groups.
- If one or more index cases of *E. leei* were to occur in the wild, establishment and spread would be less likely than in aquarium facilities because the densities of susceptible animals are much less which reduces the opportunities for transmission.
- The likelihood of *E. leei* in a wild population spreading to its natural geographic limits is greater than for other hazards with limited host ranges, such as *Aeromonas salmonicida salmonicida* (typical *A. salmoncidia*).
- If *E. leei* were to establish in the wild, especially in waters around aquaculture facilities, it may spread to facilities through wild infected fish or less likely via water.

- Spread of *E. leei* from the wild to the aquarium industry may occur in cases of subclinically affected species being collected from the wild for sale by the aquarium industry, but any clinically affected fish are unlikely to be harvested from the wild.

Conclusion
Based on these considerations and using the descriptors in Table 3, the partial likelihood of establishment and spread of *E. leei* in each exposure group for the outbreak scenario was estimated to be:

- Australian ornamental fish—**Low**.
- Farmed foodfish—**Very low**.
- Wild fish—**Very low**.

Determining adverse impacts resulting from the outbreak scenario
The factors considered relevant when determining the adverse impacts resulting from establishment and spread of *E. leei* included:

Direct effects

The effect on the life or health (including production effects) of susceptible animals and fish

- *E. leei* causes high mortalities in farmed fish but none of those species are farmed in Australia. Losses due to *E. leei* would be significant for the marine ornamental industry.
- *E. leei* may impact wild fisheries in Australia. There are reports of *E. leei* in wild fish overseas but not associated with mortalities.
- Based on the impacts of *E. leei* infection overseas, *E. leei* establishment and spread in Australia would be expected to cause minor impacts at the district or region level on the life or health of susceptible species.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There is a wide host range for *E. leei* present in Australia.
- Infection with *E. leei* has been reported to cause serious effect in wild fish populations overseas, but with no associated mortalities.
- The direct impact of *E. leei* establishment and spread on the living environment is expected to be minor at the local level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with *E. leei* is not listed as a notifiable disease by WOAH and is not included on Australia's *National list of reportable diseases of aquatic animals*. However, state and territory governments would still be expected to report on the presence of an unlisted agent that has never been reported in Australia.
- If *E. leei* was confined to an ornamental facility, then attempts at eradication may be undertaken.
- To demonstrate that eradication is successful, there would need to be a surveillance exercise over at least two years to confirm freedom, at considerable cost.

- If a movement restricted area were put in place for *E. leei*, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- If *E. leei* was confirmed in the environment, the inherent difficulties for the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating *E. leei* from wild populations is unlikely to be undertaken.
- Eradication and control of *E. leei* is expected to cause minor impacts at the district or region level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Movement restriction areas put in place would have impacts on industries supplying inputs into the affected regions. For example, where aquaculture production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- Infected fish may show clinical signs which would affect their marketability.
- *E. leei* establishment and spread would likely have a minor impact at the district or region level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Whist infection with *E. leei* is not WOAH-listed disease, importing countries may still have import requirements for live, fresh or frozen species susceptible to *E. leei*.
- If *E. leei* were to become established, Australia could use zoning to maintain access to international markets for live susceptible species.
- The impacts of *E. leei* establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- *E. leei* has a wide host range and has been reported in wild fish.
- No endangered Australian fish species are currently known to be susceptible to *E. leei*.
- An *Epinephelus* species is susceptible to *E. leei*. If *E. leei* were to cause disease in *Epinephelus daemelii* (black rock cod), it could have an impact on the survival of this already vulnerable species.
- A conservative approach has been adopted in light of the susceptibility of native species.
- The impacts of *E. leei* establishment and spread on environmental biodiversity is likely to be minor at the district or region level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Fish susceptible to *E. leei* are attractive for diving and could be affected by mortalities and movement restriction areas put in place which may impact on social amenity.

- In local areas where ornamental trade is a major industry, an *E. leei* outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of *E. leei* establishment and spread are expected to be minor at the district or region level.

Table 12 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of *E. leei*. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section Determining impacts for detailed methodology).

Table 12 Overall impact of establishment and spread of *E. leei* for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	District or region	Minor	C
	The environment (native animals/plants, and non-living environment)	Local	Minor	B
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	District or region	Minor	C
	Economic (domestic trade effects and impact on other associated industries)	District or region	Minor	C
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	District or region	Minor	C
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	C

Conclusion
The overall impact of establishment and spread of *E. leei* was estimated to be **very low**.

6.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for *E. leei* in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 6) and found to be:

- Australia ornamental fish—**Negligible**.
- Farmed foodfish—**Negligible**.
- Wild fish—**Negligible**.

6.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of *E. leei* for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 7 and found to be:

- Australian ornamental fish— **Negligible**.
- Farmed foodfish—**Negligible**.
- Wild fish—**Negligible**.

6.3.7 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 7.

The overall annual risk associated with *E. leei* was found to be **negligible**.

Therefore, as the overall annual risk achieves Australia's ALOP, specific biosecurity measures are not considered necessary for this hazard.

7 *Megalocytivirus*

7.1 Background

The genus *Megalocytivirus*, family *Iridoviridae,* comprises two species of genetically and epidemiologically distinct viruses, the infectious spleen and kidney necrosis virus (ISKNV) and the scale drop disease virus (SDDV) (Chinchar et al. 2017a; de Groof et al. 2015; Gibson-Kueh et al. 2012). These two species have been formally classified by the International Committee on Taxonomy of Viruses (ICTV) as *Megalocytivirus pagrus 1* and *Megalocytivirus lates 1*, respectively (ICTV 2024).

Megalocytivirus pagrus 1, encompasses a collection of closely related virus strains or genogroups including (Chinchar et al. 2017a; ICTV 2024):

- ISKNV genogroup
- red sea bream iridovirus (RSIV) genogroup
- turbot reddish body iridovirus (TRBIV) genogroup.

ISKNV, RSBIV and TRBIV genogroups are the aetiological agents of severe disease associated with high mortality in a range of marine and freshwater finfish species (Chinchar et al. 2017a; Kurita & Nakajima 2012).

The more distant virus species, *Megalocytivirus lates 1*, the SDDV, is not considered a hazard in this risk review because it does not infect live marine ornamental fish currently included on the department's List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A).

The first outbreak due to *Megalocytivirus* (ISKNV species) was recorded in farmed *Pagrus major* (red sea bream) in 1990 (Inouye et al. 1992). Since then, *Megalocytivirus* has been detected in other Asian, European, North and South American countries (McGrogan et al. 1998; Rodger et al. 1997; Shahin et al. 2021).

At the time of preparing this report, only infection with RSIV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (WOAH 2023b). Infection with ISKNV, RSIV and TRBIV are included on Australia's *National list of reportable diseases of aquatic animals* (AHC 2021).

ISKNV, RSIV and TRBIV are considered exotic to Australia. Australia has a long history of passive surveillance and a strong system in place to detect incursions. *Megalocytivirus* has not been recorded from wild fish in Australia. However, some have been detected in ornamental fish in quarantine at the international border, and in retail pet shops (Australian Government Department of Agriculture 2019; Mohr et al. 2015; Nolan et al. 2015; Rimmer et al. 2015). Also, ISKNV genotype associated with disease was detected in farmed *Maccullochella peelii* (Murray cod) in Victoria in 2003 (Go et al. 2006; Lancaster, Williamson & Schroen 2003), which was subsequently eradicated (Department of Agriculture 2014).

7.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of *Megalocytivirus* is warranted.

7.2.1 Agent properties

Megalocytivirus comprises large icosahedral, double-stranded DNA (dsDNA)-containing viruses, that belong to the subfamily *Alphairidovirinae* and family *Iridoviridae* (ICTV 2022). Based on sequence analysis and serological studies, all *Megalocytivirus* isolated to date appear to be strains of two viral species, ISKNV and SDDV (Chinchar et al. 2017a; ICTV 2022). These two species have been formally classified by the International Committee on Taxonomy of Viruses (ICTV) as *Megalocytivirus pagrus 1* (ISKNV) and *Megalocytivirus lates 1* (SDDV) (ICTV 2024).

Megalocytivirus pagrus 1 includes the three genogroups ISKNV, RSIV and TRBIV (ICTV 2024). These genogroups present differences in genomic sequence but display infection with the same clinical signs, histopathology, and epidemiology in susceptible fish species (Chao et al. 2002; Chao et al. 2004; Chen, Lin & Wang 2003; Chinchar et al. 2017a; Gibson-Kueh et al. 2004; Jeong et al. 2008a; Jeong et al. 2008b; Kim et al. 2010; Rimmer et al. 2015; Rimmer et al. 2016; Rodger et al. 1997; Sudthongkong, Miyata & Miyazaki 2002b, a; Weber et al. 2009; WOAH 2022c).

Megalocytivirus lates 1 (the SDDV species), on the other hand, has a low nucleotide identity and presents different pathology in susceptible fish to the ISKNV species (Chinchar et al. 2017a; de Groof et al. 2015; Gibson-Kueh et al. 2012; ICTV 2024; WOAH 2022c).

Members of the ISKNV, RSIV and TRBIV genogroups cause disease associated with mortality in a broad range of marine and freshwater finfish species (listed in section 7.2.2 Host range). They are and named based on the species that they were first detected in (Bloch & Larsen 1993; Chinchar et al. 2017a; Go & Whittington 2019; Johan & Zainathan 2020; OIE 2021e; Vazquez-Sauceda et al. 2016).

The survival of *Megalocytivirus* outside a host is unknown (Fusianto, Hick & Becker 2019; OIE 2021e), but survivability appears to be environment (seawater and freshwater), time and temperature dependent. Experimentally, members of the ISKNV genogroup have shown to be stable and remain infectious in seawater and freshwater at 25°C for at least 48–72 hours. *Oplegnathus fasciatus* (rock bream) intraperitoneally injected with an ISKNV-tissue homogenate (pearl gourami iridovirus) incubated at 25°C in freshwater or seawater for 0, 1, 2, 3 or 4 days showed mortalities of more than 60% when injected with the homogenate incubated in freshwater after 4 days. In contrast, no cumulative mortality was induced in fish injected with the ISKNV incubated in saltwater after 4 days, while the ISKNV incubated for 1–3 days in saltwater induced 100% cumulative mortality within 2 weeks (Jeong et al. 2008a). ISKNV-contaminated aquarium water, held at 25°C, was demonstrated to remain infectious to naïve Murray cod for at least 48 hours after ISKNV-diseased fish were removed (Fusianto, Hick & Becker 2019). In seawater, a reduction in the RSIV titre was observed to decrease quicky to below the detection limit (<2.0 $TCID_{50}$/mL)) within 24 hours after incubation at 25°C (Ito et al. 2013b). In contrast, the titre of RSIV in seawater only decreased to under the detection limit at day 4 when incubated at 20°C, and at day 7 when incubated at 15°C (Ito & Olesen 2013). Together, these results suggest that RSIV would not remain infectious in seawater outside of

the host for long at warmer temperatures but could survive at colder temperature for a longer period (Ito et al. 2013b).

In general, *Megalocytivirus* is inactivated by heating at 56°C for 30 min (Chinchar et al. 2017a). RSIV has been shown to be sensitive to heat treatment at 56°C for 30 min (Kurita & Nakajima 2012; Nakajima & Sorimachi 1994). ISKNV has been effectively inactivated by heating at 65°C for 20 min (Fusianto, Hick & Becker 2019).

RSIV has been shown to be sensitive to chloroform and ether treatment, formalin (0.1%) and pH 3 (Kurita & Nakajima 2012; Nakajima & Sorimachi 1994; OIE 2021e). ISKNV has been shown to be sensitive to pH 3, pH 11, 1% Virkon™, 1000 ppm sodium hypochlorite and benzalkonium chloride treatment (Fusianto, Hick & Becker 2019).

7.2.2 Epidemiology

Host range

Megalocytivirus has a wide host range (Chinchar et al. 2017b). The WOAH ad hoc *Group on susceptibility of fish species to infection with WOAH listed diseases* (WOAH ad hoc group) undertook assessments of species to determine their susceptibility to *Megalocytivirus* (excluding SDDV). The host range of *Megalocytivirus*, in accordance with the *Report of the WOAH ad hoc group on susceptibility of fish species to infection with WOAH listed diseases* (WOAH 2022c) is presented in full in Appendix C. The report can be found in the WOAH webpage (WOAH 2022c). Table 13 presents a summary of the host range of *Megalocytivirus*, which include species identified by the WOAH ad hoc group and species reported in other scientific publications. Table 13 presents a summary, it is important to note that in some cases not all species in the genera listed are reported as susceptible.

Table 13 Genera susceptible to *Megalocytivirus*

Environment	Family	Genus	Permitted import	*Megalocytivirus* (excluding SDDV)	ISKNV genogroup		RSIV genogroup		TRBIV genogroup
				Susceptible to infection	Susceptible to infection	Positive PCR; no active infection	Susceptible to infection	Positive PCR; no active infection	Susceptible to infection
Marine	Apogonidae	*Cheilodipterus*	Yes	-	-	N	-	-	-
		Ostorhinchus	Yes	-	-	N	-	-	-
		Pterapogon	Yes	Y	N	N	-	-	-
	Epinephelidae	*Cephalopholis*	No	-	-	N	-	-	-
	Ephippidae	*Platax*	Yes	N	N	-	N	-	-
		Parapristipoma	No	N	-	-	N	-	-
		Plectorhynchus	No	N	-	-	N	-	-
	Girellidae	*Girella*	No	N	-	-	N	-	-
	Lethrinidae	*Leiognathus*	No	-	-	N	-	N	-
		Lethrinus	No	N	-	-	N	-	-
	Lophiidae	*Lophiomus*	No	-	N	-	-	-	-
	Moronidae	*Lateolabrax*	No	-	-	N, E	N	-	-
	Mugilidae	*Mugil*	No	N	-	-	N	-	-
	Oplegnathidae	*Oplegnathus*	No	N	N, E	-	N	-	N
	Paralichthyidae	*Paralichthys*	No	N	-	-	-	-	N
	Pleuronectidae	*Eopsetta*	No	-	N	-	-	-	-
		Verasper	No	N	-	-	-	-	N
	Pomacentridae	*Amphiprion*	Yes	-	-	N	-	N	-
		Chromis	Yes	-	-	N	-	-	-
	Rachycentridae	*Rachycentron*	No	N	-	-	N	-	-

Environment	Family	Genus	Permitted import	*Megalocytivirus* (excluding SDDV)	ISKNV genogroup		RSIV genogroup		TRBIV genogroup
				Susceptible to infection	Susceptible to infection	Positive PCR; no active infection	Susceptible to infection	Positive PCR; no active infection	Susceptible to infection
	Scatophagidae	*Scatophagus*	No	-	N	-	-	-	-
	Sciaenidae	*Argyrosomus*	No	-	-	N	-	-	-
		Larimichthys	No	N	N	-	N	-	-
		Otolithes	No	-	-	N	-	-	-
		Sciaenops	No	N	N	-	-	-	-
	Scombridae	*Scomber*	No	N	-	-	N	-	-
		Scomberomorus	No	N	-	-	N	-	-
		Thunnus	No	N	-	-	N	-	-
	Scophthalmidae	*Scophthalmus*	No	N	-	-	-	-	N
	Scorpaenidae	*Pterois*	Yes	-	-	-	-	N	-
	Sebastidae	*Sebastes*	No	-	-	-	N[I]	-	-
	Scorpididae	*Scorpis*	No	-	E	-	-	-	-
	Serranidae	*Cromileptes*	Yes*	-	-	-	E[I]	-	-
		Epinephelus	No	N	N	N	N	-	-
	Sparidae	*Acanthopagrus*	No	N	-	N	N	-	-
		Dentex	No	N					
		Pagrus	No	N	-	N	N	-	-
		Rhabdosargu	No	-	-	-	N[I]	-	-
	Sphyraenidae	*Sphyraena*	No	-	N	-	-	-	-
	Stromateidae	*Pampus*	No	N	-	-	N	-	-
	Synanceiidae	*Inimicus*	No	N	N	-	-	-	-
Euryhaline	Latidae	*Lates*	n/a	-	N	-	N	-	-

Environment	Family	Genus	Permitted import	*Megalocytivirus* (excluding SDDV)	ISKNV genogroup		RSIV genogroup		TRBIV genogroup
				Susceptible to infection	Susceptible to infection	Positive PCR; no active infection	Susceptible to infection	Positive PCR; no active infection	Susceptible to infection
	Percalatidae	*Macquaria*	n/a	-	E	-	-	-	-
	Tetraodontidae	*Takifugu*	No	N	-	-	-	-	-
Freshwater	Aplocheilidae	*Aplocheilichthys*	n/a	-	N, E	-	-	-	
	Butidae	*Oxyeleotris*	n/a	N	N	-	N	-	-
	Cyprinidae	*Epalzeorhynchos*	n/a	N	N	-	-	-	-
		Carassius	n/a	-	-	N	-	-	-
		Cyprinus	n/a	-	-	N	-	-	-
	Carangidae	*Caranx*	n/a	-	N	-	-	-	-
		Pseudocaranx	n/a	N	-	-	N	-	-
		Seriola	n/a	N	-	-	N, E	-	-
		Trachinotus	n/a	N	-	-	N	-	-
		Trachurus	n/a	N	-	-	N	-	-
	Centrarchidae	*Lepomis*	n/a	N	-	-	N	-	-
		Micropterus	n/a	-	E[I]	-	-	-	-
	Cichlidae	*Astronotus*	n/a	-	N	-	-	-	-
		Etroplus	n/a	N	N	-	-	-	-
		Laetacara	n/a	-	N	-	-	-	-
		Microgeophagus	n/a	-	N	-	-	-	-
		Oreochromis	n/a	N	N	-	-	-	
		Pelvicachromis	n/a	-	N	-	-	-	-
		Pterophyllum	n/a	N	N	-	-	-	-
	Danionidae	*Danio*	n/a	N	N	-	-	-	-

Environment	Family	Genus	Permitted import	*Megalocytivirus* (excluding SDDV)	ISKNV genogroup		RSIV genogroup		TRBIV genogroup
				Susceptible to infection	Susceptible to infection	Positive PCR; no active infection	Susceptible to infection	Positive PCR; no active infection	Susceptible to infection
	Nothobranchiidae	*Aphyosemion*	n/a	N	N	-	-	-	-
	Osphronemidae	*Betta*	n/a	N[I]	-	-	-	-	-
		Macropodus	n/a	N	-				
		Osphronemus	n/a	N	N				
		Trichogaster	n/a	N	N	-	-	-	-
		Trichopodus	n/a	N	N, E	-	-	-	-
	Percichthyidae	*Maccullochella*	n/a	N	E	-	-	-	-
		Nannoperca	n/a	-	E	-	-	-	-
	Plotosidae	*Tandanus*	n/a	-	-	E	-	-	-
	Poeciliidae	*Poecilia*	n/a	N	N	-	-	-	-
		Xiphophorus	n/a	N	N	-	-	-	-
	Procatopodidae	*Poropanchax*	n/a	N	-	-	-	-	-
	Serrasalmidae	*Metynnis*	n/a	-	N[I]	-	-	-	-
	Sinipercidae	*Siniperca*	n/a	N	-	-	N, E	-	-

Permitted import Included on the department's List of permitted live marine ornamental fish suitable for import. **n/a** Not applicable, these species are not included on the department's List of permitted live marine ornamental fish suitable for import and are outside the scope of this review. Yes* *Cromileptes* spp. (excluding *Cromileptes altivelis*). **N** Susceptible to infection by natural exposure. **E** Susceptible to infection by experimental exposure. **I** Incomplete evidence for susceptibility.

Megalocytivirus has been reported to affect most fish life stages. RSIV and viruses in the RSIV genogroup have been reported to affect juveniles to adults, with juveniles being more susceptible (WOAH 2023h). Infection with ISKNV and viruses in the ISKNV genogroup have been reported to affect larvae, fingerlings, juveniles and adult groupers (Chao et al. 2004).

Geographical distribution

Megalocytiviral disease was first reported in the early 1990s in farmed red sea bream in Japan and caused by RSIV (Inouye et al. 1992; OIE 2021e). Since then, RSIV and other *Megalocytivirus* have been reported in Asia, mainly in fish from East and Southeast Asia (Kurita & Nakajima 2012; Weng et al. 2002). There are some few reports of *Megalocytivirus* detections in Europe and the Americas (McGrogan et al. 1998; Shahin et al. 2021), including detections in ornamental fish (Rodger et al. 1997; United States Department of Agriculture 2022).

Prevalence

Marine ornamental fish

In 2015, *Megalocytivirus* was detected in 92% (n=12) of consignments of freshwater and marine ornamental fish collected immediately upon arrival in Australia and under quarantine. From the consignments, a total of 62 populations of ornamental fish were collected, including 46 populations of marine ornamental fish (Becker et al. 2017). Approximately 52% (n=46) of the populations of fish tested for *Megalocytivirus* were positive. The populations that tested positive included the species *Amphiprion sebae* (sebae anemonefish)*, Cheilodipterus quinquelineatus* (five-lined cardinalfish)*, Chromis viridis* (blue green damselfish)*, Ostorhinchus compressus* (ochre-striped cardinalfish) and *Pterapogon kauderni* (Banggai cardinalfish) (Becker et al. 2017).

Other marine fish

There are few published surveys of *Megalocytivirus* in wild populations of fish. A survey of wild marine fish harvested in the southern coastal area of Korea from 2010–12 detected RSIV in 0.4% (n=253) of the fish samples. The fish tested were indigenous species in Korean and Japan coastal areas including rock bream (Kim et al. 2013). The average prevalence of ISKNV genogroup viruses in marine fish collected in the South China Sea (wild and cultured) between November 2001 and January 2003 was 14.6% (n=1666) (Wang et al. 2007). The marine fish collected represented 6 orders, 25 families, and 86 species of fish (Wang et al. 2007). The annual prevalence was higher in cultured fish (25.7%, n=548) that in wild fish (9.1%, n=1118) (Wang et al. 2007).

Prevalence rates of *Megalocytivirus* in farmed fish have been reported. Red sea bream iridoviral disease (RSIVD) is considered prevalent in farmed fish populations (OIE 2021e). ISKNV was detected, by qPCR at a prevalence of 0–13% (n=30 fish per farm) in juvenile *Epinephelus* spp. (grouper) from farms (5 out of 7) surveyed from November 2014 to December 2015 in Indonesian nurseries on the east coast of Aceh (Sah Putra et al. 2020). Co-infection with nervous necrosis virus was reported from all farms (Sah Putra et al. 2020). Prevalence rates of *Megalocytivirus* of 12.1% were reported in farmed fish from Damietta and Sharkia Governorates, Egypt (Mallah, Mansour & Tarabily 2021).

Mortalities

Mortalities due to *Megalocytivirus* depend on host species and age, water temperature, and environmental conditions (Australian Government Department of Agriculture 2019; WOAH 2023h).

Marine ornamental fish

Mortalities of wild-caught Banggai cardinalfish of up to 100% due to RSIV genogroup (Banggai cardinalfish iridovirus), have been reported in breeding facilities shortly after obtaining the fish from wholesalers (Weber et al. 2009).

Other fish

Mortalities in farmed fish due to megalocitivirus infection have been reported. Mortality of approximately 70% due to RSIV was reported in cultured juvenile red sea bream in Japan (Nakajima et al. 1999; Nakajima et al. 1998). Mortalities estimated to be 60% due to RSIV were reported in cultured rock bream in the Korean peninsula during August to September 1998 (Kim et al. 2002). During this time, RSIV also caused mortalities of <5% in red seabream (Kim et al. 2002). Mortality of up to 75% due to RSIV genogroup (large yellow croaker iridovirus) were reported in cultured *Larimichthys crocea* (large yellow croaker) in China during the summers of 1999–2001 (Chen, Lin & Wang 2003). Mortalities up to 70% due to ISKNV genogroup have also been reported in *Sciaenops ocellatus* (red drum) (15–17 cm body length) cultured in net-cages in coastal areas of China (Weng et al. 2002). Mortality due to ISKNV genogroup (grouper iridovirus in Taiwan) infection in cultured groupers from Taiwan has been reported to reach 60–80% in juveniles and 10–30% in larger fish (Chao et al. 2004). Mortalities up to 60–80% due to ISKNV genogroup were reported in cultured marine groupers (*Epinephelus coioide, E. malabaricus* and *E. awoara*), red drum, and large yellow croaker (Wang et al. 2007). An outbreak of disease due to TRBIV was reported to cause mortalities between 50–70% in juvenile turbot at aquaculture farms in Korea (Kim et al. 2005).

In freshwater fish, mortality rates of 90% in Murray cod fingerlings were reported in an aquaculture facility and attributed to ISKNV genogroup (Murray cod iridovirus) (Go et al. 2006; Go & Whittington 2006; Lancaster, Williamson & Schroen 2003)

Transmission

The natural mode of transmission of *Megalocytivirus* may be primarily horizontal through direct contact with virus contaminated water or via cohabitation with infected fish (Rimmer et al. 2016; Sudthongkong, Miyata & Miyazaki 2002a; WOAH 2023h). Experimentally, horizontal transmission via cohabitation, water, ingestion of excreta or cannibalism has been achieved (Australian Government Department of Agriculture 2019; Jeong et al. 2008a; Rimmer et al. 2016; Sudthongkong, Miyata & Miyazaki 2002a). *Megalocytivirus* is shed into the water from infected fish (Rimmer et al. 2016). Transmission of *Megalocytivirus* from broodstock to progeny has not been investigated (WOAH 2023h).

Megalocytivirus infect freshwater, brackish, and marine fish hosts. It has been shown that hosts can transmit *Megalocytivirus* across freshwater and marine environments using euryhaline species (Go & Whittington 2019). For example, an ISKNV genotype (dwarf gourami iridovirus) was experimentally transmitted via cohabitation from freshwater Murray cod to euryhaline *Macquaria novemaculeata* (Australian bass) and then to marine *Scorpis lineolata* (Silver sweep) (Go & Whittington 2019). In addition, the ISKNV genotype was successfully transmitted from the marine silver sweep to the euryhaline Australian bass and back again to the freshwater Murray cod (Go & Whittington 2019).

There is evidence that some species may be long-term asymptomatic carriers of ISKNV (Wang et al. 2007).

Mechanism of spread

The mechanism of spread of *Megalocytivirus* into new countries and/or areas has been attributed to the international trade of ornamental and foodfish species (Go et al. 2006; Go et al. 2016; Ramírez-Paredes et al. 2021; Rimmer et al. 2016; Wang et al. 2007; Weber et al. 2009). For example, *Megalocytivirus* has been detected in consignments of ornamental fish imported into Australia and held under quarantine at the international border and in retail pet shops, where infections have been quickly contained and eradicated. (Becker et al. 2017; Go et al. 2006; Rimmer et al. 2015). Feeding infected aquarium fish to broodstock has also been suggested as a means of spreading *Megalocytivirus* (Lancaster, Williamson & Schroen 2003).

Infectious dose

The minimum infectious dose of *Megalocytivirus* required to cause disease in susceptible species by experimental challenge or natural infection is unknown. However, immersion of African lampeye in freshwater containing a RSIV genogroup suspension (African lampeye iridovirus) at 10^5 $TCID_{50}$/mL or at 10^7 $TCID_{50}$/mL for 2 hours, resulted in 100% cumulative mortality by 12–14 days (Sudthongkong, Miyata & Miyazaki 2002a). Immersion of red sea bream in water containing RSIV at $10^{2.7}$ and $10^{0.8}$ $TCID_{50}$/mL resulted in 90% and 75% mortality of the challenged fish, respectively (Jung et al. 1997)((Ito et al. 2013a) citing (Tanaka et al. 2003). Intraperitoneal inoculation of the marine silver sweep with 50µL of ISKNV genogroup (dwarf gourami iridovirus) inoculum containing 1×10^7 copies of virus resulted in severe morbidity and 62.5% mortality by 17 days post-inoculation (Rimmer et al. 2016). Intraperitoneal injection of fish with 50µL of ISKNV genogroup (dwarf gourami iridovirus) inoculum containing approximately 2×10^5 copies/mg of tissue resulted in clinical signs, histological lesions consistent with ISKNV infection and cumulative mortalities of 100% in *Macquaria ambigua* (Golden perch) and *Macquaria australasica* (Macquarie perch), 90% in Murray cod and 55% in *Nannoperca australis* (Southern pygmy perch) (Rimmer et al. 2016).

7.2.3 Pathogenesis

Water temperature has been reported to be a major factor influencing the outbreaks of *Megalocytivirus* (Wang et al. 2007). Outbreaks due to RSIV have been seen mostly in the summer season at water temperatures of 25°C and above (OIE 2021e). An epizootic due to RSIV genogroup (large yellow croaker iridovirus) in cultured large yellow croaker was reported to occur at seawater temperature between 27–30°C (Chen, Lin & Wang 2003). Acute infection and mortalities by ISKNV genogroup had been observed to occur at temperatures of 20–32°C (Wang et al. 2007). It has been suggested that fish species that are latently infected may serve as carriers of ISKNV genogroup at temperatures below 20°C (Wang et al. 2007). Outbreaks of TRBIV disease has been reported at temperatures of approximately 17–18°C (Kim et al. 2005).

Tissue tropism

Infection with *Megalocytivirus* is mainly observed in the spleen, kidney, liver, heart, gill and the digestive tract (Chinchar et al. 2017a; Jung et al. 1997; Kim et al. 2005; OIE 2021e). ISKNV genogroup has also been reported in brain tissue (Weng et al. 2002).

Tissue titre

Following intraperitoneal inoculation of silver sweep with ISKNV genogroup, surviving fish had viral copy numbers of 1.57×10^2–4.31×10^3 copies/mg tissue 28 days post-inoculation (Go & Whittington 2019). Silver sweep that died during the trial reported viral copy numbers of 2.60×10^6–1.14×10^8 copies/mg tissue. Cohabitation of naïve silver sweep with experimentally ISKNV-infected

silver sweep resulted in 100% mortality by 13 days post-exposure. Challenged fish had viral copy numbers of 4.15×10^5–6.10×10^8 copies/mg tissue (Go & Whittington 2019).

7.2.4 Diagnosis

Clinical signs

Clinical signs of RSIVD include lethargy, petechiae in the gills and enlargement of the spleen (WOAH 2023h).

Gross signs of disease caused by ISKNV genogroup include changes in body colour (darkening or lightening), exophthalmos (popeye) and abdominal distension (due to fluid or enlargement of organs) (Australian Government Department of Agriculture 2019; Sudthongkong, Miyata & Miyazaki 2002a; Wang et al. 2007). Splenomegaly and pale gills have also been reported (Sudthongkong, Miyata & Miyazaki 2002a), along with loss of equilibrium, lateral recumbency and non-responsiveness to stimulation (Go & Whittington 2019). In the marine silver sweep, intraspecific aggression (tattered fins and loss of scales), inappetence, depigmentation and tachybranchia (increased rate of respiration) have been observed (Go & Whittington 2019). ISKNV genogroup have also been detected by PCR in a wide range of fish with no clinical signs of disease (Jeong et al. 2008b; Rimmer et al. 2015; Wang et al. 2007). Experimental trials (infection by intraperitoneal inoculation or bath exposure) have shown that clinical signs of infection and mortality due to ISKNV genogroup usually begin about 7–10 days after exposure (Go & Whittington 2006; He et al. 2002; Weber et al. 2009).

Clinical signs of disease caused by TRBIV in fish include pale body colour, protruding eyes, enlarged abdomen, spleen and kidney and pale gills and/or liver (Kim et al. 2005).

Megalocytivirus have been detected in susceptible ornamental fish species without showing clinical signs of disease (Go & Whittington 2006).

Pathology

Systemic formation of many inclusion body bearing cells (IBCs) are observed in the spleen, the lamina propria of the intestines, kidney, eye, liver and gills during infection (Go & Whittington 2019; Kim et al. 2005; Sudthongkong, Miyata & Miyazaki 2002a). Electron microscopic examination of mature IBCs revealed the inclusion bodies are composed of virions, rough endoplasmic reticula, smooth endoplasmic reticula, mitochondria and abundant ribosomes within a marginally compressed cytoplasm (Sudthongkong, Miyata & Miyazaki 2002a). Necrosis of splenic cells, renal parenchyma, myocardium and hematopoietic cells are also reported (Kim et al. 2005; Sudthongkong, Miyata & Miyazaki 2002a; Weber et al. 2009; Weng et al. 2002).

Testing

Chapter 2.3.7 of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH Manual) provides details of the methods currently available for targeted surveillance and diagnosis of RSIV (WOAH 2023h). PCR, sequencing and immunofluorescent antibody test (IFAT) are the recommended methods for presumptive and confirmatory diagnosis of RSIV (WOAH 2023h). Virus isolation followed by IFAT or PCR are the recommended methods for targeted surveillance of RSIV (WOAH 2023h).

The use of monoclonal antibody M10 is described in the WOAH Manual and is applicable to RSIV and ISKNV (Kurita & Nakajima 2012; WOAH 2023h).

The Australian and New Zealand Standard Diagnostic Procedures (ANZSDP) describes methods of *Megalocytivirus* detection for confirmation as the cause of disease in clinically affected fish or for surveillance purposes (Crane & Moody 2018). ANZSDP uses quantitative PCR and sequencing for surveillance and diagnostic testing for *Megalocytivirus*. Conventional PCR assays designed to detect and differentiate genogroups of the *Megalocytivirus* are also available.

7.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments.

An effective formalin-killed commercial vaccine for RSIVD is currently available for red sea bream, striped jack (*Pseudocaranx dentex*), *Epinephelus malabaricus* (Malabar grouper), *Epinephelus coioides* (orange-spotted grouper) and fish species belonging to the genus *Seriola* in Japan (WOAH 2023h).

7.2.6 Control and prevention

Methods to control *Megalocytivirus* mainly rely on avoiding exposure to the virus by introducing pathogen-free fish, coupled with good hygiene practices on farms, and avoiding practices that can decrease water quality and/or increase stress, such as overcrowding and overfeeding (WOAH 2023h).

7.2.7 Impact of the disease

Megalocytivirus causes mass mortalities in marine and freshwater fish in Asian countries (Go & Whittington 2006; Wang et al. 2009; Wang et al. 2007). *Megalocytivirus* is responsible for losses that threaten the production and economic sustainability of important cultured fish species (McGrogan et al. 1998; Ramírez-Paredes et al. 2021; Shahin et al. 2021).

It has been reported that a single barramundi farm in Vietnam lost income estimated up to US$435,810/year due to ISKNV infection (Dong et al. 2017). During an ISKNV outbreak in tilapia in Ghana, losses of more than 10 tonnes/day were recorded from many farms due to mortality rates of 60–90% (Ramírez-Paredes et al. 2021). ISKNV genogroup (grouper iridovirus in Taiwan) infection has caused high economical loss in grouper cultured in Taiwan and southeast Asia (Chao et al. 2004).

Production losses due to high mortalities (about 90%) over a period of several weeks of 10,000 Murray cod fingerlings (4-6 cm) were reported in an aquaculture facility in Victoria in 2003 and attributed to the ISKNV genogroup (Murray cod iridovirus) (Go et al. 2006; Lancaster, Williamson & Schroen 2003). The virus was subsequently eradicated (Department of Agriculture 2014).

7.2.8 Current biosecurity measures

There are no current biosecurity measures specific for *Megalocytivirus* in marine ornamental fish species.

Megalocytivirus was considered in the *Import risk analysis on live ornamental fish* released in 1999 (Ornamental fish IRA) (AQIS 1999). At the time, a risk assessment was conducted for marine-species-specific iridoviruses, including RSIV, and the overall risk was determined to achieve Australia's appropriate level of protection (ALOP). Therefore, risk management measures were not recommended.

The risk of ISKNV and ISKNV genogroup in freshwater fish species was also considered in the *Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami*

iridovirus and related viruses conducted in 2014 (Gourami iridovirus review) (Department of Agriculture 2014). The overall risk of *Megalocytivirus* did not achieve Australia's ALOP and risk management measures were recommended. In addition to the general requirements for freshwater fish, the following *Megalocytivirus* specific measures were recommended for susceptible species; gouramis, bettas, paradise fish, cichlids and poeciliids:

- sourcing from *Megalocytivirus*-free populations
- batch testing for *Megalocytivirus*.

7.2.9 Conclusion

Megalocytivirus, including virus species ISKNV and its genogroups, ISKNV, RSIV and TRBIV, are present in exporting countries, are not present in Australia and are capable of causing adverse effects. In Australia, infection with RSIV, ISKNV and TRBIV are nationally notifiable diseases. Based on the preceding information, risk assessment is warranted.

7.3 Risk assessment

Based on chapter 2 and the technical information about *Megalocytivirus* including virus species ISKNV and its genogroups, ISKNV, RSIV and TRBIV presented in this chapter, the risk assessment was completed.

A summary of the risk assessment values are shown in Appendix D.

7.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for *Megalocytivirus* were that:

- This risk review is generic and therefore the entry assessment assumes that *Megalocytivirus* is present in all source countries.
- There are reports of *Megalocytivirus* infecting marine ornamental fish on the *Permitted marine species list*. *Pterapogon kauderni* (Banggai cardinalfish), which belongs to the Apogonidae family, and *Platax orbicularis* (orbiculate batfish), which belongs to the Ephippidae family, are susceptible to ISKNV genogroup (Sriwanayos et al. 2013; Weber et al. 2009; WOAH 2022c).
- Prevalence of *Megalocytivirus* in marine ornamental fish can be up to 50%.
- *Megalocytivirus* can survive in seawater for 1–3 days at 25°C (Fusianto, Hick & Becker 2019; Ito et al. 2013b; Jeong et al. 2008a).
- Infected imported fish would likely carry sufficient *Megalocytivirus* to cause infection in susceptible species.
- Pre-export inspection may detect ornamental fish showing clinical signs of infection with *Megalocytivirus* and remove them before export. Fish sub-clinically infected or carrier fish would not be identified through visual inspection.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 3, the annual likelihood of entry of *Megalocytivirus* was estimated to be **moderate**.

7.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for *Megalocytivirus* were that:

- *Megalocytivirus* can be transmitted horizontally by cohabitation, cannibalism, ingestion of faeces or infected tissue, and waterborne contamination.
- *Megalocytivirus* are estimated to survive and remain infectious for up to 3 days in seawater (Jeong et al. 2008a).
- *Megalocytivirus* would be expected to be present in sufficient loads in imported fish to cause infection in susceptible species if exposed.
- Species susceptible to *Megalocytivirus* infection are present in Australia. Marine susceptible species include barramundi and the marine groupers (WOAH 2022c). Silver sweep, which is also reported to be susceptible, are found in great abundance in shallow waters around reefs in New South Wales (NSW) and are commonly captured by both commercial and recreational fishers (Go & Whittington 2019). The euryhaline Australian bass, the barramundi cod and *Scatophagus argus* (spotted butterfish) have also been reported to be susceptible (Chao 2003; Go & Whittington 2019; Mahardika et al. 2004).
- RSIV and ISKNV typically cause infection and mortalities in susceptible fish at water temperatures of 20–25°C and above (Wang et al. 2007; WOAH 2023h). TRBIV disease has been reported at temperatures of about 17–18°C (Kim et al. 2005).
- Because of the holding conditions in aquarium facilities, any ornamental susceptible species grown with, or sharing the same water as infected imported fish, will likely be exposed to viable *Megalocytivirus*.
- Farmed foodfish in Australia known to be susceptible to *Megalocytivirus* include barramundi, yellowtail kingfish, groupers and Murray cod. It is unlikely farmed fish would be in the vicinity of the shore where imported live marine ornamental fish would be released or that they could make their way to a fish cage. However, farmed fish near estuaries and coastal lagoons are more likely to be exposed. Feeding imported marine ornamental fish to farmed foodfish broodstock is assumed to be unlikely because of their high cost and the large volume and specific nutritional profile that farmed foodfish broodstock require.
- Introduction into the wild may occur by direct release of imported marine ornamental fish or their associated waste/water into natural waters. This would be a direct pathway to wild susceptible species. However, the survival of marine ornamental fish released into the wild is expected to be reduced because of their environmental sensitivity.
- For hazards with a wide host range such as *Megalocytivirus*, the likelihood of wild susceptible fish encountering that hazard is greater in comparison to those hazards with a smaller host range. However, wild susceptible species would likely be more scattered compared to the dense populations found in aquaculture, which would reduce opportunities for transmission.

Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 3, the partial likelihood of exposure of each exposure group to *Megalocytivirus* was estimated to be:

- Australian ornamental fish—**High**.

- Farmed foodfish—**Extremely low**.
- Wild fish—**Low**.

7.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to *Megalocytivirus* was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Australian ornamental fish—**Moderate**.
- Farmed foodfish— **Extremely low**.
- Wild fish—**Low**.

7.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)

The key points considered relevant when determining the partial likelihood of establishment and spread for *Megalocytivirus* were that:

- *Megalocytivirus* can be transmitted horizontally.
- *Megalocytivirus* are estimated to survive and remain infectious for up to 3 days in seawater and at least 4 days in freshwater (Jeong et al. 2008a).
- It is expected that susceptible species which come into contact with megalocytivirus-infected fish would receive an infectious dose.
- Fish that survive *Megalocytivirus* infection may become carriers and sources of the virus (Wang et al. 2007).
- *Megalocytivirus* can infect fish species present in Australian freshwater and marine environments.
- Farmed foodfish in Australia are known to be susceptible to *Megalocytivirus* (including barramundi, yellowtail kingfish, groupers, cod).
- *Megalocytivirus* could establish in ornamental susceptible species. This is due to the stressors associated with aquariums. For example, the higher density of susceptible animals and the growing conditions.
- *Megalocytivirus* are notifiable fish diseases in Australia and in Australian states and territories including Victoria, New South Wales, Tasmania, Western Australia and South Australia.
- Each state and territory have translocation protocols for aquaculture animals, which typically includes consideration of *Megalocytivirus*, which would reduce the likelihood of spread between farmed foodfish populations. Though the movement of ornamental fish between hobbyists and retail premises is difficult to monitor and not necessarily captured under all state and territory legislations.
- If *Megalocytivirus* were to establish and spread in ornamental facilities supplying a significant part of the hobby sector or many breeders, it could spread widely within this exposure group.
- It is unlikely that *Megalocytivirus* would spread from ornamental facilities to wild populations via escaped fish. Small numbers of live marine ornamental fish would not survive in the wild due to

their environmental sensitivity, and anticipated propagule pressure which would require large numbers to be released at the same time and location.

- It is unlikely that *Megalocytivirus* will spread from Australian ornamental fish to farmed foodfish due to the lack of exposure pathways and biosecurity practices expected to be in place in facilities which farm susceptible species.
- It is unlikely that *Megalocytivirus* will spread from Australian ornamental facilities to wild populations via escaped or released fish, given their environmental sensitivity and the anticipated propagule pressure required to ensure establishment of a population.
- If *Megalocytivirus* were to establish in farmed foodfish populations it could spread to wild populations through release of water from farms into natural waters. The spread in marine environments may be limited as it is unlikely that *Megalocytivirus* survives in the environmental for more than 3 days (Jeong et al. 2008a). The spread would also be moderated by dilution effects and implementation of biosecurity measures. Spread from cage operated farmed foodfish to wild fish would be more likely due to the sharing of water.
- Spread of a *Megalocytivirus* from foodfish facilities to the aquarium industry is unlikely given the closed systems and lack of exposure pathway between the two exposure groups.
- If one or more index cases of a *Megalocytivirus* were to occur in the wild, establishment and spread would be less likely than in aquarium and foodfish facilities because the densities of susceptible animals are much less, which reduces the opportunities for transmission. However, for hazards with a wide host range such as *Megalocytivirus*, the likelihood of wild susceptible fish encountering that hazard is greater in comparison to those hazards with a smaller host range.
- If *Megalocytivirus* were to establish in the wild, it may spread to cage operated farmed foodfish. It may also spread to pond or tank system operated farmed foodfish that take water from coastal or estuary environments, however the likelihood of spread is reduced because of the semi-closed nature of the systems, stronger biosecurity procedures and water treatment in place for in these facilities.
- If *Megalocytivirus* were to establish in the wild, especially in waters around aquaculture facilities, it may spread to facilities through water intake due to megalocytivirus being transmissible through water. The spread in marine environments may be moderated as it is unlikely that megalocytivirus survive in the environmental for more than 3 days.
- *Megalocytivirus* are expected to survive in freshwater environments for longer. In the absence of effective biosecurity measures, wild infected fish may be transferred into the farms through the inlet water channels.

Conclusion

Based on these considerations and using the descriptors in Table 3, the partial likelihood of establishment and spread of *Megalocytivirus* in each exposure group for the outbreak scenario was estimated to be:

- Australian ornamental fish—**Moderate**.
- Farmed foodfish—**Moderate**.
- Wild fish—**Moderate**.

Determining adverse impacts resulting from the outbreak scenario

The factors considered relevant when determining the adverse impacts resulting from establishment and spread of *Megalocytivirus* include:

Direct effects

The effect on the life or health (including production effects) of susceptible animals and fish

- *Megalocytivirus* cause mortalities in farmed fish. Losses due to *Megalocytivirus* would be significant for the marine and freshwater ornamental industry.
- *Megalocytivirus* can cause significant mortality to farmed fish, including barramundi, Murray cod, grouper, kingfish and snapper. Barramundi is one of the main aquaculture fish species in Australia. Murray cod is an emerging aquaculture species in Australia (Rimmer et al. 2016).
- There are reports of *Megalocytivirus* in wild fish but no reports associated with mortalities in the wild were found.
- Based on the impacts of megalocytivirus infection overseas, *Megalocytivirus* establishment and spread in Australia would be expected to cause minor impacts at the national level on the life or health of susceptible species.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- *Megalocytivirus* have a wide host range and there are reports of infection in wild fish populations overseas but no associated mortalities.
- The direct impact of *Megalocytivirus* establishment and spread on the living environment is expected to be minor at the state or territory level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with RSIV is listed as a notifiable disease by WOAH. Infection with ISKNV and TRBIV are included on Australia's *National list of reportable diseases of aquatic animals*. State and territory governments would be expected to report on their presence.
- If infection with *Megalocytivirus* was confined to an ornamental or aquaculture facility, then attempts at eradication may be undertaken.
- If infection with *Megalocytivirus* was confirmed in the wild, the inherent difficulties for the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating *Megalocytivirus* from wild populations is unlikely to be undertaken. Containment and control via zoning/compartmentalisation, would be the more likely control strategy.
- If a movement restriction area were put in place for an outbreak of *Megalocytivirus*, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- Eradication and control of *Megalocytivirus* is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Movement control orders, if put in place, would have indirect impacts on other industries such as ornamental fish suppliers due to the host range of *Megalocytivirus*.
- Infected fish may show clinical signs which would affect their marketability.
- *Megalocytivirus* establishment and spread would likely have a minor impact at the national level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Infection with RSIV is WOAH-listed disease. Importing countries may have import requirements or have closed borders to the import of fish products susceptible to *Megalocytivirus* to avoid the possible introduction.
- If *Megalocytivirus* were to become established, Australia could use zoning to maintain access to international markets for live susceptible species.
- The impacts of *Megalocytivirus* establishment and spread on international trade are likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- *Megalocytivirus* has a wide host range and has been reported in wild fish.
- *Megalocytivirus* is reported to produce mortality in Murray cod and Macquarie perch, which are listed as nationally vulnerable and endangered, respectively. There are other species of fish in the same family, Percichthyidae, found in Australia, including golden perch, which is vulnerable in the state of Victoria. These species are also cultured for restocking purposes or farming.
- Some *Epinephelus* species are susceptible to *Megalocytivirus*. If *Megalocytivirus* was to cause disease in *Epinephelus daemelii* (black rock cod), it could have a significant impact on the survival of this already vulnerable species.
- A conservative approach has been adopted in light of the susceptibility of native species, particularly those that are vulnerable, endangered or threatened.
- The impacts of *Megalocytivirus* establishment and spread on environmental biodiversity is likely to be minor at the national level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Movement restriction areas or area closures put in place, may have an impact on social amenity.
- In local areas where ornamental fish or aquaculture is a major industry, a *Megalocytivirus* outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of *Megalocytivirus* establishment and spread are expected to be minor at the state or territory level.

Table 14 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of *Megalocytivirus*. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section Determining impacts for detailed methodology).

Table 14 Overall impact of establishment and spread of Megalocytivirus for the outbreak scenario.

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Minor	E
	The environment (native animals/plants, and non-living environment)	State or territory	Minor	D
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	National	Minor	E
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	National	Minor	E
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	State or territory	Minor	D

Conclusion
The overall impact of establishment and spread of *Megalocytivirus*s was estimated to be **moderate**.

7.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for *Megalocytivirus* in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 6) and found to be:

- Australian Ornamental fish—**Moderate**.
- Farmed foodfish—**Moderate**.
- Wild fish—**Moderate**.

7.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of *Megalocytivirus* for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 7 and found to be:

- Australian Ornamental fish—**Moderate**.
- Farmed foodfish—**Negligible**.
- Wild fish—**Low**.

7.3.7 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 7.

The overall annual risk associated with *Megalocytivirus* was found to be **Moderate**. Therefore, as the overall annual risk does not achieve Australia's ALOP, specific biosecurity measures are considered necessary for these hazards.

7.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for *Megalocytivirus* in imported live marine ornamental fish to a level that achieves Australia's ALOP are shown in Appendix D.

Sections 7.4.1, 0, and 7.4.2 present the factors considered and the conclusions reached.

7.4.1 Source population requirements

Sourcing from a country, compartment or zone free of Megalocytivirus

When determining if sourcing from populations free from *Megalocytivirus*, would reduce the overall risk of *Megalocytivirus* to achieve Australia's ALOP, the key points considered were:

- *Megalocytivirus* have been reported in Asia (Inouye et al. 1992; Kurita & Nakajima 2012; OIE 2021e; Weng et al. 2002), Europe and the Americas (McGrogan et al. 1998; Rodger et al. 1997; Shahin et al. 2021; United States Department of Agriculture 2022)
- Determination of *Megalocytivirus* freedom would need to be to a standard consistent with that recommended for WOAH listed diseases, or equivalent.
- Sourcing from populations free from *Megalocytivirus* would reduce the likelihood of entry of *Megalocytivirus*.

Conclusion

Based on this information, the overall restricted risk of imported live marine ornamental fish with the biosecurity measure, sourcing from free populations applied, was determined to be **negligible**.

Therefore, as the overall restricted risk achieves Australia's ALOP, additional specific biosecurity measures are not considered necessary for this hazard.

Removal of susceptible species from the permitted list

When determining if removal of species susceptible to *Megalocytivirus* from the permitted list, would reduce the overall risk of *Megalocytivirus* to achieve Australia's ALOP, the key points considered were:

- Sourcing from free populations could include import of species not known to be susceptible to *Megalocytivirus*. At the time of preparing this report, *Pterapogon kauderni* (Banggai cardinalfish) and *Platax orbicularis* (orbiculate batfish) are the only species of marine ornamental fish (included on the *Permitted marine species list*) that meet the criteria for listing as susceptible to *Megalocytivirus* by WOAH (WOAH 2022c).
- Removal of species that are known to be susceptible to *Megalocytivirus* from the *Permitted marine species list* would reduce the likelihood of entry of *Megalocytivirus*. Where a whole genus is permitted import, measures would apply to all species in the genus unless there was evidence that not all species were susceptible.

Conclusion
Based on this information, the overall restricted risk of imported live marine ornamental fish with the biosecurity measure, sourcing from free populations applied, was determined to be **negligible**.

Therefore, as the overall restricted risk achieves Australia's ALOP, additional specific biosecurity measures are not considered necessary for this hazard.

7.4.2 Batch testing for *Megalocytivirus*

When determining if batch testing (pre-export or on-arrival) would reduce the overall risk of *Megalocytivirus* to a level which achieves Australia's ALOP, the key points considered were:

- There are PCR methods available to detect *Megalocytivirus* (Crane & Moody 2018).
- Testing may be applied pre-border (pre-export) or on-arrival (at border).
- Batch testing of marine ornamental fish is expected to reduce the likelihood of entry of *Megalocytivirus*, but not completely remove it. The level of protection provided by testing would depend on the availability of effective tests (including with respect to their sensitivity and commercial availability, as well as sampling and other operational procedures).
- Batch testing would only need to be applied to species known to be susceptible to *Megalocytivirus*. At the time of preparing this report, *Pterapogon kauderni* (Banggai cardinalfish) and *Platax orbicularis* (orbiculate batfish) are the only species of marine ornamental fish (included on the *Permitted marine species list*) that meet the criteria for listing as susceptible to *Megalocytivirus* by WOAH (WOAH 2022c). Where a whole genus is permitted import, measures would apply to all species in the genus unless there was evidence that not all species were susceptible.

Conclusion
Based on this information, the overall restricted risk of imported live marine ornamental fish with the biosecurity measure, batch testing (pre-export or on-arrival) for *Megalocytivirus* applied, was determined to be **very low**.

Therefore, as the overall restricted risk achieves Australia's ALOP, additional specific biosecurity measures are not considered necessary for this hazard. This means that there is no need to apply a combination of pre-border and on-arrival batch testing, either manages risk to a level which achieves Australia's ALOP.

8 Nervous necrosis virus

8.1 Background

Nervous necrosis virus (NNV) is the aetiological agent of viral nervous necrosis (VNN), also known as viral encephalopathy and retinopathy (VER) (Munday, Kwang & Moody 2002). NNV is within the genus *Betanodavirus* in the family *Nodaviridae* (Schneemann et al. 2005). There are 4 major genotypes of NNV (Nishizawa et al. 1997):

- barfin flounder nervous necrosis virus (BFNNV)
- red-spotted grouper nervous necrosis virus (RGNNV)
- striped jack nervous necrosis virus (SJNNV)
- tiger puffer nervous necrosis virus (TPNNV).

NNV reassortants combining genomic segments from RGNNV and SJNNV have also been identified (Olveira et al. 2009; Panzarin et al. 2012). Recently, a new virus named seahorse nervous necrosis virus (SHNNV) was identified from cultured *Hippocampus abdominalis* (big-belly seahorses) in Fujian Province, China but it is not clear if it is a new NNV genotype or a RGNNV strain (Chen et al. 2022).

BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV are the only genotypes that comply with the criteria described in the World Organisation for Animal Health *Aquatic animal health code* (WOAH Code) Article 2.1.2 *Hazard Identification* (WOAH 2023a) and have been retained as hazards.

VER was first described at the end of the 1980s and has since caused mortalities and serious economic losses in various marine and freshwater fish, both wild and farmed (Bandín & Souto 2020; Doan et al. 2017). It has been reported in Asia, Europe, North America and the United Kingdom (Bandín & Souto 2020; Munday, Kwang & Moody 2002).

VER is no longer listed as a disease notifiable to WOAH due to the widespread distribution of NNV (OIE 2021c). However, VER is on Australia's *National list of reportable diseases of aquatic animals* (AHC 2021). Australia has a long history of passive surveillance and a strong system in place to detect incursions. RGNNV is present in Australia (Moody et al. 2009) but BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV are considered exotic to Australia.

8.2 Technical information

8.2.1 Agent properties

NNV is an icosahedral, non-enveloped virus, approximately 25–35 nm in diameter, with 2 single-stranded, positive-sense RNA genomic segments (Mori et al. 1992; Munday, Kwang & Moody 2002). NNV is classified by the International Committee on Taxonomy of Viruses as a member of the genus *Betanodavirus*, in the family *Nodaviridae* (Schneemann et al. 2005).

NNV genotypes show different optimal growth temperatures in cell culture (15–20°C for BFNNV, 20°C for TPNNV, 20–25°C for SJNNV and 25–30°C for RGNNV) and natural infections can also occur at different water temperatures (Bandín & Souto 2020; Iwamoto et al. 2000). BFNNV has been reported to cause disease at temperatures as low as 4–15°C (Bandín & Souto 2020; Grotmol, Bergh & Totland 1999; Nylund et al. 2008) and SJNNV and SJNNV/RGNNV at 20–25°C (Souto et al. 2015; Toffan et al.

2016; Totland et al. 1999). RGNNV/SJNNV were shown to cause a persistent infection in *Solea senegalensis* (Senegalese sole) at low temperature (16°C) for over 2 months, but when temperature increased (22°C) the virus was able to trigger an acute infection and cause high mortalities (Souto, Olveira & Bandin 2015). This suggests that increases in temperature can induce subclinical infections into clinical disease.

NNV genotypes also show differences in host susceptibility (Souto et al. 2015; Toffan et al. 2016; Totland et al. 1999). For example, in challenge trials, SJNNV was highly virulent to larvae of *Pseudocaranx dentex* (striped jack) but replication was not detected in larvae of *Hippoglossus hippoglossus* (Atlantic halibut). Conversely, BFNNV that was highly virulent to the Atlantic halibut larvae did not replicate in striped jack larvae (Totland et al. 1999).

RGNNV is relatively stable in the environment, retaining infectivity for at least 6 months in seawater and 3 months in freshwater at 15°C under experimental conditions (Frerichs et al. 2000). No specific information is available on stability of BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV but they are assumed to be similar.

NNV survives freezing as stocks stored at –20°C (Nishioka et al. 2016) and –80°C (Arimoto et al. 1993; Grotmol, Bergh & Totland 1999; Iwamoto et al. 2000; Ma et al. 2015; Souto, Olveira & Bandin 2015) could induce infections when used in challenge studies. Virions are sensitive to sodium hypochlorite, calcium hypochlorite, benzalkonium chloride, iodine, ethanol, methanol, high pH, UV irradiation and ozone (Arimoto et al. 1996; Frerichs et al. 2000). SJNNV is inactivated by heating at 60°C for 30 minutes (Arimoto et al. 1996).

8.2.2 Epidemiology

Host range

Species which are susceptible to infection with BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV are summarised in Table 15 and presented in full in Appendix C.

Table 15 Genera susceptible to NNV

Environment	Family	Genus	Permitted import	NNV		Genotype
				Susceptible to infection	Positive PCR; no active infection	
Marine	Acanthuridae	*Zebrasoma*	Yes	-	N	BFNNV
	Carangidae	*Carangoides*		-	N	SJNNV
		Pseudocaranx	n/a	N	-	SJNNV
		Selene	n/a	-	N	NNV*
		Seriola	No	-	N	SJNNV
		Trachurus	No	N, E	N	SJNNV, RGNNV/SJNNV
	Centriscidae	*Aeoliscus*	Yes	-	N	NNV*
	Gadidae	*Gadus*	n/a	N	-	BFNNV
		Melanogrammus	No	N	-	BFNNV
	Labridae	*Ctenolabrus*	Yes	-	N	BFNNV

Environment	Family	Genus	Permitted import	NNV		Genotype
				Susceptible to infection	Positive PCR; no active infection	
		Labrus	Yes	-	N	BFNNV
		Symphodus	Yes	-	N	BFNNV
	Monocentridae	*Monocentris*	Yes	-	N	BFNNV
	Muraenidae	*Rhinomuraena*	Yes	-	N	BFNNV
	Paralichthyidae	*Paralichthys*	No	N	-	BFNNV, TPNNV
	Pleuronectidae	*Hippoglossus*	No	N	-	BFNNV
		Pseudopleuronectes	No	N	-	BFNNV
		Verasper	No	N	-	BFNNV
	Scombridae	*Scomber*	No	-	N	SJNNV
	Scophthalmidae	*Scophthalmus*	n/a	N	-	BFNNV
	Sparidae	*Evynnis*	No	-	N	SJNNV
		Pagus	No	-	N	SJNNV
		Sparus	n/a	N	-	SJNNV, BFNNV RGNNV/SJNNV
	Soleidae	*Solea*	No	N	-	SJNNV, BFNNV, RGNNV/SJNNV
	Pomacentridae	*Dascyllus*	Yes	-	N	NNV*
Euryhaline	Anguillidae	*Anguilla*	n/a	-	N	SJNNV
	Chanidae	*Chanos*	n/a	-	N	NNV*
	Moronidae	*Dicentrarchus*	n/a	N, E	-	BFNNV, SJNNV, RGNNV/SJNNV
	Salmonidae	*Salmo*	n/a	E	-	BFNNV
	Sciaenidae	*Argyrosomus*	n/a	-	N	SJNNV
	Tetraodontidae	*Takifugu*	n/a	N	-	TPNNV

Permitted import Included on the department's List of permitted live marine ornamental fish suitable for import. **n/a** Not applicable, these species are not included on the department's List of permitted live marine ornamental fish suitable for import and are outside the scope of this review. **N** Susceptible to infection by natural exposure. N^{I} incomplete evidence for susceptibility. **E** Susceptible to infection by experimental exposure.

*Species that have been shown to be susceptible to NNV, without genotype testing to confirm strain, only have 'NNV' listed under the genotype column.

NNV infects all life stages of fish, especially larvae and juveniles (Bandín & Souto 2020; Munday, Kwang & Moody 2002). Host susceptibility to NNV is influenced by multiple factors including virus strain, fish species, age, and life stage (Bandín & Souto 2020; Munday, Kwang & Moody 2002).

There are reports of NNV infecting permitted species of marine ornamental fish. Wild fish from the family Labridae (wrasse) collected across Swedish and Norwegian coasts (Korsnes et al. 2017) and marine fish stocked in a commercial aquarium in Seoul, the Republic of Korea (Gomez et al. 2006) tested positive for NNV via RT-PCR. No clinical signs of disease were observed in the fish sampled suggesting the wrasse and ornamental fish may act as carriers for NNV (Gomez et al. 2006; Korsnes et al. 2017). BFNNV, RGNNV and unidentified NNV genotypes were detected, indicating that a variety

of NNV genotypes are naturally present in permitted species of marine ornamental fish. NNV was detected in two populations of *Pterapogon kauderni* (Banggai cardinal fish) and one of *Zoramia leptacantha* (Threadfin cardinalfish) that were imported to Australia from Indonesia though the genotypes were not identified further (Becker et al. 2017).

Geographical distribution

BFNNV seems to be limited to cold-water fish in Canada, China, Japan, the Republic of Korea, United Kingdom (UK), United States of America and Northern areas of Europe (e.g. France, Norway and Sweden) (Bandín & Souto 2020; Kim et al. 2018; Munday, Kwang & Moody 2002).

SJNNV has been reported in China, Italy, Japan, Portugal and Spain (Cutrín et al. 2007; Ma et al. 2015; Mori et al. 1992; Thiery, Cozien & de Boisseson 2004).

SJNNV/RGNNV has only been isolated in Greece (Athanassopoulou et al. 2003), whereas the opposite form, RGNNV/SJNNV, is widespread in Southern Europe including in Croatia, Cyprus, Greece, Italy, Portugal and Spain (Olveira et al. 2009; Panzarin et al. 2012).

TPNNV has only been described in one fish species in Japan (Nishizawa et al. 1997).

Prevalence

Marine ornamental fish

NNV was detected by RT-PCR in 4.5% (n=177) of apparently healthy marine fish collected from a commercial aquarium in Seoul, the Republic of Korea, in 2005–06 (Gomez et al. 2006). Wild wrasse species, *Ctenolabrus rupestris* (rock cook wrasse)*, Symphodus melops* (corkwing wrasse), and *Labrus bergylta* (ballan wrasse)*,* surveyed from the Norwegian and Swedish coasts in 2014 tested positive for NNV at a prevalence of 6.7% (n=466) (Korsnes et al. 2017). Species prevalence was 6.4% (n=343) in rock cook wrasse, 6.3% (n=112) in corkwing wrasse, and 18% (n=11) in ballan wrasse. In total, 18 wrasse NNV strains were genotyped as either BFNNV or RGNNV and 13 strains could not be genotyped.

Other fish

Prevalence data on BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV or TPNNV infections is low, likely due to the acute mortality associated with the disease and that these strains are less common compared to RGNNV. Healthy, wild *Trachurus japonicus* (Japanese jack mackerel) caught in two Japanese coastal areas in 2003 (Miyako) and 2005 (Saiki) tested positive for SJNNV at a prevalence of 6% (n=62) and 48% (n=92), respectively (Nishioka et al. 2016). An investigation of healthy, wild fish collected from surrounding ocean areas of Japan in 2003–2005 reported SJNNV at a prevalence of 4.8% (n=729) across 6 species (Sakamoto et al. 2008). SJNNV was detected in 47% (n=32) of healthy, wild *Argyrosomus regius* (meagre) caught in the Iberian Peninsula (Lopez-Jimena et al. 2010). A survey of wild *Anguilla anguilla* (European eel) collected in Spain in 2004 and 2008 found 6% (n=62) and 40% (n=117) were positive for SJNNV, respectively (Bandín et al. 2014).

Live shellfish collected in the Republic of Korea during 2011–2014 tested positive for BFNNV at a prevalence of 26.3% (n=741) (Kim, Cuenca & Olesen 2018).

Mortalities

Mortality due to NNV infection is variable and the disease outcome is influenced by several factors, particularly the age and species of the host fish (Arimoto, Maruyama & Furusawa 1994; Bloch,

Gravningen & Larsen 1991; Grotmol et al. 1997; Mori, Mushiake & Arimoto 1998; Munday, Kwang & Moody 2002; Watanabe, Nishizawa & Yoshimizu 2000). High mortality is typically observed in larval and juvenile stages whereas lower losses have generally been reported in older fish (Munday, Kwang & Moody 2002; WOAH 2019).

Marine ornamental fish

No reports of mortalities in marine ornamental fish due to NNV were found.

Other fish

SJNNV-positive striped jack spawners produced larvae that were SJNNV-positive within 3–7 days post-hatch (dph) with cumulative mortalities of 70–100% within 10 dph (Nishizawa, Muroga & Arimoto 1996). An SJNNV infection killed all 400 million larvae of striped jack in a fish farm in Japan (Arimoto et al. 1993). SJNNV infecting farmed Senegalese sole in Spain in 2003 caused severe mortalities (no numbers given) (Thiery, Cozien & de Boisseson 2004).

In Norway, acute high mortality nearing 100% occurred in multiple commercial hatcheries and juvenile rearing facilities for Atlantic halibut due to BFNNV infections (Grotmol et al. 1997; Johansen et al. 2004; Johansen et al. 2002). Farmed *Dicentrarchus labrax* (European seabass), in both recirculating and fresh open water production facilities in Greece, suffered NNV outbreaks in 2000 reaching mortalities of 30% (Athanassopoulou et al. 2003). Outbreaks of BFNNV in farmed *Gadus morhua* (Atlantic cod) in the UK in 2000 resulted in mortalities of approximately 2% over a 3-month period (Starkey et al. 2001). BFNNV infection in Senegalese sole fingerlings resulted in almost 100% mortality in a population of approximately 10,000 over a few months (Starkey et al. 2001). In 2006, BFNNV infection was diagnosed in Atlantic cod kept in sea cages in Parisvatn, Norway that caused an estimated 10–15% mortality (Patel et al. 2007). A mortality rate >90% due to BFNNV infection was observed in a *Gadus macrocephalus* (Pacific cod) larvae-rearing facility in China (Mao et al. 2015).

In 2014, *Sparus aurata* (gilthead bream) broodstock showing no clinical signs of NNV introduced to a farm in Europe produced larvae that suffered 80–98% mortality starting from 17 dph that was attributed to RGNNV/SJNNV (Toffan et al. 2017). In 2015, a RGNNV/SJNNV outbreak in 50–70 dph larvae of a separate gilthead bream farm in Europe caused a cumulative mortality of 60% (Toffan et al. 2017). In an Italian hatchery, multiple RGNNV/SJNNV outbreaks during 2017–18 in gilthead bream and European seabass caused 10% and 100% mortality, respectively (Volpe et al. 2020).

Transmission

NNV can be transmitted horizontally fish to fish, through water or via contaminated equipment (Arimoto et al. 1993; Grotmol, Bergh & Totland 1999; Korsnes et al. 2012; Nguyen, Nakai & Muroga 1996; Souto et al. 2015; Souto, Olveira & Bandin 2015; WOAH 2019). Vertical transmission has been highly suspected in some species as NNV has been frequently detected in the ovaries and testes of spawners (Mao et al. 2015; Mushiake et al. 1994; Nguyen et al. 1997; Nguyen, Nakai & Muroga 1996; Nishizawa, Muroga & Arimoto 1996; Watanabe, Nishizawa & Yoshimizu 2000), in eggs (Mao et al. 2015), fertilised eggs (Arimoto et al. 1992; Grotmol & Totland 2000; Mao et al. 2015) and larvae up to 10 dph (Arimoto et al. 1992; Mori, Mushiake & Arimoto 1998; Mushiake et al. 1994; Nguyen et al. 1997; Nguyen, Nakai & Muroga 1996).

Surviving fish can become carriers of the virus for extended periods and may be able to transmit the infection to other fish (Arimoto, Maruyama & Furusawa 1994). For example, several studies on SJNNV in striped jack suggest that larvae are primarily infected with the virus through broodstock showing no clinical signs (Arimoto et al. 1992; Mori, Mushiake & Arimoto 1998; Mushiake et al. 1994; Nguyen et al. 1997; Nguyen, Nakai & Muroga 1996). Following a natural infection, infectious RGNNV/SJNNV could still be detected in survivor gilthead bream 6–7 months later (Toffan et al. 2017). Similarly, BFNNV could be re-isolated from Atlantic halibut one year after a natural NNV outbreak (Johansen et al. 2004). Stress factors such as increases in temperature, repeated spawning, high stocking density, feed quality and water quality can induce a carrier into clinical disease (Arimoto et al. 1993; Johansen et al. 2004; Mushiake et al. 1994; Souto, Olveira & Bandin 2015).

NNV has been isolated from bivalve molluscs (e.g. mussel, clam and oysters), rotifers and some crustaceans, suggesting they may act as natural reservoirs or possible carriers of the virus (Bandín & Souto 2020; Kim et al. 2018). *Artemia salina* (brine shrimp) and *Brachionus plicatilis* (common rotifer), which are used as live food for marine fish larvae, became NNV-positive after bath exposure to SJNNV and RGNNV/SJNNV (Skliris & Richards 1998; Vazquez-Salgado et al. 2020). Vazquez-Salgado et al (2020) further showed that brine shrimp were capable of transmitting RGNNV/SJNNV to Senegalese sole larvae (via feeding), that resulted in clinical signs and high mortality (Vazquez-Salgado et al. 2020).

Mechanism of spread

The introduction of NNV into new areas has been primarily attributed to the movement of live animals. Many studies have reported the virus in wild marine fish species, including valuable fish used as feed for marine aquaculture (e.g. mackerel), suggesting that wild fish could be a possible source of the virus in infections of cultured fish (Nishioka et al. 2016; Sakamoto et al. 2008). In aquaculture facilities, NNV may be introduced through the collection of subclinical broodstock from wild populations (Munday, Kwang & Moody 2002).

Infectious dose

In an immersion challenge, 1-day-old larvae of striped jack and Japanese jack mackerel exposed to 10^7 $TCID_{50}$/mL SJNNV for 1 hour resulted in 90–100% mortality at 3–9 days post infection (dpi) (Nishioka et al. 2016). Injection of striped jack (mean weight 84 g) with 5×10^6 $TCID_{50}$/mL SJNNV induced an infection but did not cause any clinical signs or mortality in the fish (Banu et al. 2007). Senegalese sole juveniles (mean weight 2 g) infected by bath exposure for 3 hours using 10^5 $TCID_{50}$/mL SJNNV or RGNNV/SJNNV started dying from 5 dpi and reached 100% mortality at 18–25 dpi (Souto et al. 2015). European seabass (mean weight 0.2 g) challenged by bath exposure to 10^4 $TCID_{50}$/mL SJNNV, RGNNV/SJNNV and SJNNV/RGNNV strains for 2 hours resulted in clinical signs of infection and mortality (Vendramin et al. 2014).

8.2.3 Pathogenesis

Tissue tropism

NNV has a tropism for cells of the spinal cord, brain and retina (Grotmol et al. 1997; Johansen et al. 2002; Mori et al. 1992; Nguyen, Nakai & Muroga 1996). However, NNV has also been detected in the heart, intestine, liver, spleen, kidney, stomach, epithelium, gills, fins, ovaries, testes and fertilised eggs (Arimoto et al. 1992; Grotmol et al. 1997; Korsnes et al. 2009; Nguyen et al. 1997; Nguyen, Nakai & Muroga 1996; Souto et al. 2018).

Tissue titre

The titres of SJNNV in dead larvae following an immersion challenge were $10^{6.0}$–$10^{10.3}$ $TCID_{50}$/g for striped jack and $10^{4.0}$–$10^{7.6}$ $TCID_{50}$/g for Japanese jack mackerel (Nishioka et al. 2016). SJNNV intramuscularly injected into striped jack (mean weight 84 g) reached a maximum titre of $10^{7.9}$ $TCID_{50}$/g in the spinal cord at 5 dpi, $10^{6.9}$ $TCID_{50}$/g in the brain at 7 dpi, $10^{5.1}$ $TCID_{50}$/g in the eye at 14 dpi and $10^{8.2}$ $TCID_{50}$/g in the kidney at 5 dpi (Banu et al. 2007). In bath challenge experiments of European seabass (mean weight 2g) with SJNNV, although no clinical signs or mortalities were observed at 30 dpi, virus was still detected in the nervous tissue (brain and eyes) of survivor fish at 2.4×10^8 RNA copies/g and 1.6×10^7 $TCID_{50}$/g (Souto et al. 2015).

Immersion challenge of Senegalese sole juveniles (mean weight 1 g) with SJNNV and RGNNV/SJNNV resulted in virus titres being detected in the brain at 1 dpi with 1.1×10^4 and 9.4×10^4 RNA copies/g, respectively, that reached 2.8×10^8 and 2.4×10^9 RNA copies/g, respectively, by 15 dpi. The SJNNV and RGNNV/SJNNV infective viral titres in the brain at 1–2 dpi were 1.7×10^2 and 2.4×10^2 $TCID_{50}$/g, respectively and by 15 dpi, had increased to 5.6×10^6 and 5.6×10^7 $TCID_{50}$/g, respectively (Souto et al. 2018). Viral particles were also detected in the gills, skin, fins and intestine. The highest SJNNV RNA copy numbers were 1.3×10^6 copies/g in the skin at 12 dpi, 2.8×10^3 copies/g in fins at 14 dpi, 1.2×10^4 copies/g in gills at 15 dpi and 1.0×10^3 copies/g in intestines at 1 dpi. For RGNNV/SJNNV, the highest RNA copy numbers were 4.1×10^5 copies/g in skin at 12 dpi, 1.6×10^5 copies/g in fins at 12 dpi, 1.5-2.2 $\times 10^6$ copies/g in gills at 11–13 dpi and $>10^5$ copies/g in intestines at 1 dpi (Souto et al. 2018). In bath challenged Senegalese sole (mean weight 2 g), the viral titres in dead fish was highest at 6.9×10^5 $TCID_{50}$/g at 10–13 dpi for fish infected with RGNNV/SJNNV and at 7.4×10^4 $TCID_{50}$/g at 15–20 dpi for SJNNV (Souto et al. 2015).

BFNNV was detected at 10^7 copies/ng total RNA from brain tissue of naturally infected Pacific cod showing clinical signs (Mao et al. 2015). The BFNNV copy number in Pacific cod eggs ranged from <10–10^3 copies/ng total RNA (Mao et al. 2015).

8.2.4 Diagnosis

Clinical signs

Abnormal swimming behaviour (e.g. spiral swimming, whirling, horizontal looping or darting) and loss of appetite are commonly observed among affected fish. Other clinical signs can include lethargy, swim bladder hyperinflation and coloration abnormalities (pale or dark) (Bandín & Souto 2020; Munday, Kwang & Moody 2002). In many cases, especially for larvae and juveniles, the only sign of infection is mortality (WOAH 2019). In other cases, a subclinical infection can develop (Athanassopoulou et al. 2003; Bitchava et al. 2019; Johansen et al. 2004; Johansen et al. 2002; Nguyen et al. 1997).

Pathology

Histopathological analysis of infected fish typically show vacuolation, necrosis and degeneration of nervous cells of the spinal cord, brain and/or retina (Arimoto et al. 1993; Grotmol et al. 1997; Johansen et al. 2004; Nguyen, Nakai & Muroga 1996). These lesions are more prominent in larvae and juveniles while in older fish they are sometimes very rare and difficult to detect (WOAH 2019). There are some reports of lesions characterised by necrotic cells and the presence of cytoplasmic vacuoles in additional tissues, including the heart, gills, intestine and epithelium, but such lesions are not consistently reported (Grotmol et al. 1997; Nguyen, Nakai & Muroga 1996).

Testing
Virus isolation by cell culture (Frerichs, Rodger & Peric 1996; Iwamoto et al. 2000; Moody & Crane 2014) followed by immunological or molecular identification is traditionally used to diagnose NNV infections (Doan et al. 2017). Immunological methods include indirect fluorescent antibody test, immunohistochemistry and enzyme linked immunosorbent assay (Arimoto et al. 1992; Moody & Crane 2014; Nguyen, Nakai & Muroga 1996; Watanabe, Nishizawa & Yoshimizu 2000). RT-PCR is the most rapid and convenient molecular method for diagnosing clinically infected fish (Bigarré et al. 2010; Dalla Valle et al. 2000; Grotmol et al. 2000; Johansen et al. 2002; Nishizawa et al. 1994) while nested RT-PCR (Dalla Valle et al. 2000) or real-time RT-PCR (Baud et al. 2015; Dalla Valle et al. 2005; Panzarin et al. 2010) are useful tools for diagnosing subclinically infected or carrier fish (WOAH 2019).

8.2.5 Treatment
There is currently no effective treatment available for NNV (WOAH 2019).

8.2.6 Control and prevention
Prevention of the disease is primarily by good husbandry and biosecurity to avoid exposure of the farmed population to the virus (WOAH 2019). For example, stocking with NNV-negative fish, selection of NNV-free broodstock for spawning, using virus-free water, avoiding use of NNV-infected fish as feed for aquaculture, drying and disinfecting hatching facilities between batches of larvae (Bandín & Souto 2020; Munday, Kwang & Moody 2002). The virus can be completely inactivated by means of chemical disinfectants such as sodium hypochlorite, calcium hypochloride, benzalkonium chloride, chloroquine and iodine or by other chemical (ozone) or physical treatments (heat, UV light) (Arimoto et al. 1996; Frerichs et al. 2000). Washing fertilised eggs in ozone-treated sea water has been effective in the control of the disease (Arimoto et al. 1996; Grotmol & Totland 2000; Mori, Mushiake & Arimoto 1998; Watanabe, Nishizawa & Yoshimizu 2000). However, disinfection of eggs is not 100% effective (Watanabe, Nishizawa & Yoshimizu 2000). It is also important to reduce stress factors such as providing adequate food for broodstock and decreasing the stocking density of larvae and juveniles (Mushiake et al. 1994).

Numerous vaccines have been designed using DNA as well as peptides, recombinant protein, inactivated NNV, subunit, live virus and virus-like particles as antigen for controlling RGNNV (Tanaka et al. 2001; Vimal et al. 2014; Yamashita et al. 2005). Two commercial inactivated (formalin-killed) vaccines directed against the RGNNV genotype, Alpha ject micro®1Noda (Pharmaq) and Icthiovac®VNN (Hipra), are available for European seabass vaccination in the Mediterranean market (Bandín & Souto 2020) and one vaccine (OceanTect VNN) is commercially available in Japan ((Kuroda & Nakai 2012) cited in (Nishioka et al. 2016)). In contrast, few vaccines, if any, have targeted SJNNV, BFNNV or TPNNV (Sommerset et al. 2005b; Souto et al. 2023). Several studies have investigated the use of antiviral compounds to control NNV infections but at present none are being actively used in aquaculture facilities (Bandín & Souto 2020).

8.2.7 Impact of the disease
NNV causes a devastating disease among cultured marine fish worldwide and results in significant economic losses to the aquaculture industry (Chi, Wu & Hong 2016). An NNV outbreak is characterised not only by massive mortalities but also by a marked reduction in fish growth and increasing differences in weight/size of the affected fish, which represents a significant cost loss that

is often underestimated (Vendramin et al. 2014). The Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN) states that an outbreak in a batch of fish in a hatchery typically results in termination of the production run on the assumption that mortality will be very high (DAWR 2017). In Europe, NNV is a major concern for farmed gilthead bream and European seabass where production in 2020 was 208 tonnes and 199,476 tonnes, respectively, in Mediterranean countries (EURL for Fish and Crustacean Diseases 2021).

8.2.8 Current biosecurity measures

There are no current biosecurity measures for NNV associated with imported live marine ornamental fish.

8.2.9 Conclusion

BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV are present in exporting countries, are not present in Australia and can cause adverse effects. In Australia, viral encephalopathy and retinopathy is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

8.3 Risk assessment

Based on chapter 2 and the technical information about NNV presented in this chapter, the risk assessment was completed. For simplicity, NNV in the risk assessment refers to the genotypes BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV unless otherwise stated. RGNNV was not retained as a hazard and is excluded from the risk assessment.

A summary of the risk assessment values are shown in Appendix D.

8.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for NNV were that:

- This risk review is generic and therefore the entry assessment assumes that NNV is present in all source countries.
- There is one report of BFNNV present in wild *Ctenolabrus rupestris* (goldsinny wrasse), *Symphodus melops* (corkwing wrasse) and *Labrus bergylta* (ballan wrasse) collected across the Norwegian and Swedish coasts. None of the sampled fish showed any clinical signs of infection (Korsnes et al. 2017). *Labridae* species, which include wrasse, are on the List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A).
- A commercial aquarium in South Korea sampled apparently healthy marine ornamental fish, with several species testing positive for NNV (genotype not identified). Of these species, *Aeoliscus strigatus* (shrimp fish), *Dascyllus trimaculatus* (three spot damsel), *Monocentris japonica* (pinecone fish), *Rhinomuraena quaesita* (blue ribbon eel) and *Zebrasoma flavesenes* (yellow tang) are on the *Permitted marine species list* (Gomez et al. 2006).
- The prevalence of NNV in wild wrasse ranged from 6-18% (Korsnes et al. 2017). The prevalence of NNV in other marine ornamental species is unknown.
- The prevalence of NNV can be up to 100% in farmed foodfish and 48% in wild foodfish populations.

- RGNNV can remain viable in water for several months (Frerichs et al. 2000). It is assumed other NNV genotypes can similarly survive in the environment for a period.
- The viral load of NNV in infected imported fish would likely be sufficient to cause infection in susceptible species.
- Pre-export inspection may detect marine ornamental fish showing clinical signs that are typical of infection with NNV and remove them before export. Live marine ornamental fish with mild, or no clinical signs, would not be identified through visual inspection.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the annual likelihood of entry of NNV was estimated to be **very low**.

8.3.2 Exposure assessment

The key points considered relevant when conducting the direct exposure assessment for NNV were that:

- NNV can be transmitted via contact between fish and through water. Vertical transmission is also suspected.
- RGNNV can remain viable in water for several months (Frerichs et al. 2000). It is assumed other NNV genotypes can similarly survive in the environment for a period.
- NNV would be expected to be present in sufficient loads in imported fish to cause infection in susceptible species if exposed.
- Species susceptible to NNV infection are present in Australia including, but not limited to, species of wrasse, *Chanos chanos* (milkfish)*, Salmo salar* (Atlantic salmon), striped jack/silver trevally*, Seriola dumerili* (greater amberjack), *Trachurus* sp. (trevally) and yellow tang.
- BFNNV has been reported to cause disease at temperatures as low as 4–15°C (Bandín & Souto 2020)(Bandín & Souto 2020; Grotmol, Bergh & Totland 1999; Nylund et al. 2008) and SJNNV and SJNNV/RGNNV at 20–25°C (Souto et al. 2015; Toffan et al. 2016; Totland et al. 1999).
- Because of the holding conditions in aquarium facilities, any ornamental susceptible species grown with, or sharing the same water as infected imported fish, will likely be exposed to viable NNV.
- Farmed foodfish in Australia are known to be susceptible to NNV (e.g. salmonids, mackerel). However, it is unlikely farmed fish would be in the vicinity of the shore where imported live marine ornamental fish would be released or that they could make their way to a fish cage. Feeding imported marine ornamental fish to farmed foodfish broodstock is assumed to be unlikely because of their high cost and the large volume and specific nutritional profile that farmed foodfish broodstock require.
- Introduction into the wild may occur by direct release of imported marine ornamental fish or their associated waste/water into natural waters. This would be a direct pathway to wild susceptible species. However, the survival of marine ornamental fish released into the wild is expected to be reduced because of their environmental sensitivity.
- NNV has a modest host range present in Australian waters that could be infected. However, wild susceptible species would likely be more scattered compared to the dense populations found in aquaculture, which would reduce opportunities for transmission.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the partial likelihood of exposure of each exposure group to NNV was estimated to be:

- Australian ornamental fish—**Very low**.
- Farmed foodfish—**Extremely low**.
- Wild fish—**Very low**.

8.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to NNV was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Australian ornamental fish—**Extremely low**
- Farmed foodfish—**Extremely low**.
- Wild fish—**Extremely low**.

8.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)
The key points considered relevant when determining the partial likelihood of establishment and spread for NNV were that:

- NNV can be transmitted via contact between fish and through water. Vertical transmission may occur.
- RGNNV can remain viable in water for several months (Frerichs et al. 2000). It is assumed other NNV genotypes can similarly survive in the environment for a period.
- It is expected that susceptible species in contact with NNV-infected fish would be exposed to virus loads sufficient for infection.
- Fish that survive NNV infections may become carriers and sources of the virus.
- Species susceptible to NNV infection are present in Australia including, but not limited to, wrassees, milkfish, Atlantic salmon, striped jack/silver trevally, greater amberjack, trevallies and yellow tang.
- BFNNV has been reported to cause disease at temperatures as low as 4–15°C and SJNNV and SJNNV/RGNNV at 20–25°C.
- There are no commercially available treatments for NNV infections.
- There is a possibility of NNV establishing and spreading in Australian ornamental fish populations. This is due to infected marine ornamental fish being carriers of NNV and the stressors associated with aquariums. For example, the high density of susceptible animals and the holding conditions. However, the likelihood is reduced by the limited range of marine ornamental fish that are susceptible to NNV infection.
- Each state and territory have translocation protocols for aquaculture animals, which typically includes consideration of NNV, which would reduce the likelihood of spread between farmed foodfish populations. Though the movement of ornamental fish between hobbyists and retail

premises is difficult to monitor and not necessarily captured under all state and territory legislations.

- It is unlikely that NNV will spread from Australian ornamental fish to farmed foodfish due to the lack of exposure pathways and biosecurity practices expected to be in place in facilities which farm susceptible species.
- It is unlikely that NNV will spread from Australian ornamental facilities to wild populations via escaped or released fish, given their environmental sensitivity and the anticipated propagule pressure required to ensure establishment of a population.
- Farmed foodfish in Australia are known to be susceptible to NNV (e.g. salmonids).
- If NNV were to establish in farmed foodfish populations it could spread to wild populations through release of water from farms into natural waters. NNV can remain viable in water for months. The spread would be moderated by dilution effects and implementation of biosecurity measures. Spread from cage operated farmed foodfish to wild fish would be even more likely due to the sharing of water.
- Spread of NNV from foodfish facilities to Australian ornamental fish is unlikely given the closed systems, lack of exposure pathway between the two exposure groups, and the limited marine ornamental fish susceptible to NNV.
- If one or more index cases of NNV were to occur in the wild, establishment and spread would be more likely than in Australian ornamental fish populations due to a wider host range of NNV being available and its effective transmission via water. The ability of fish to be subclinically infected with NNV and to remain carriers after surviving an infection would also aid spread.
- If NNV were to establish in the wild, it may easily spread to cage operated farmed foodfish due to the sharing of water and opportunities for direct fish contact.
- If NNV were to establish in the wild, especially in waters around foodfish aquaculture facilities, it may spread to facilities through water intake due to NNV being transmissible through water. The spread may be moderated through water dilution and biosecurity measures in place.
- Spread of NNV from the wild to the Australian ornamental fish population may occur in cases of subclinically affected species being collected from the wild for sale by the aquarium industry, but any clinically affected fish are unlikely to be harvested from the wild.

Conclusion

Based on these considerations and using the descriptors in Table 3, the partial likelihood of establishment and spread of NNV in each exposure group for the outbreak scenario was estimated to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish—**Moderate**.
- Wild fish—**Low**.

Determining adverse impacts resulting from the outbreak scenario

The factors considered relevant when determining the adverse impacts results in establishment and spread of NNV include:

Direct effects

The effect on the life or health (including production effects) of susceptible animals and fish

- NNV causes high mortalities in farmed fish and BFNNV can infect *Salmo salar* (Atlantic salmon). Production and productivity losses due to NNV would be significant for the Australian salmonid industry with aquaculture production valued at approximately A$1.15 billion in 2021–22 (Tuynman et al. 2023).
- NNV has not been observed to cause mortality in infected marine ornamental fish. Losses due to NNV are not anticipated to occur in, or impact, the marine ornamental fish industry.
- NNV may impact wild fisheries in Australia. There are reports of NNV in wild fish overseas although none were associated with mortalities or declines in catch rates.
- Based on the impacts of NNV infection overseas, NNV establishment and spread in Australia would be expected to cause significant impacts at the national level on the life or health of susceptible species.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- NNV can infect wild fish present in Australia.
- There are reports of infection in wild fish populations overseas. However, infection in wild fish is typically only subclinical or a carrier state.
- The direct impact of NNV establishment and spread on the living environment is expected to be minor at the district or region level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with NNV is not listed as a notifiable disease by WOAH but is included on Australia's *National list of reportable diseases of aquatic animals*. States and territories would be required to report on the occurrence of NNV.
- If NNV was confined to an aquaculture facility, then an attempt at eradication may be undertaken. The cost of an eradication attempt in affected salmonid farms would be significant for the industry.
- To demonstrate that eradication is successful, there would need to be a surveillance exercise over at least two years to confirm freedom, at considerable cost.
- If a movement restricted area were put in place for NNV, there would be ongoing costs associated with the containment, surveillance, monitoring and implementation of controls.
- If infection with NNV was confirmed in the wild, the inherent difficulties for the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradication is unlikely to be undertaken.
- Eradication and control of NNV is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Movement restriction orders, if put in place, would have indirect impacts on other industries such as seafood suppliers, commercial wild catch fisheries and bait fisheries due to the host range of NNV.
- Industries supplying inputs into the affected regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- NNV-infected fish may show clinical signs which would affect their marketability.
- NNV establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Whilst infection with NNV is not a WOAH-listed disease, importing countries may still have import requirements for live, fresh or frozen species susceptible to NNV.
- If NNV were to become established, Australia could use zoning to maintain access to international markets for live susceptible species.
- The impacts of NNV establishment and spread on international trade are likely to be minor at the state or territory level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- NNV has a moderate host range and has been reported in wild fish.
- There are no species listed as endangered in Australia that are related to species known to be susceptible to NNV.
- The impacts of NNV establishment and spread on environmental biodiversity are unlikely to be discernible at any level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Recreational fishing for susceptible species could be affected by movement restriction areas put in place due to an outbreak of NNV which may impact on social amenity.
- In local areas where aquaculture is a major industry, an NNV outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of NNV establishment and spread are expected to be minor at the minor at the state or territory level.

Table 16 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of NNV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section Determining impacts for detailed methodology).

Table 16 Overall impact of establishment and spread of NNV for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Significant	F
	The environment (native animals/plants, and non-living environment)	District or region	Minor	C
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	State or territory	Minor	D
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	A
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	State or territory	Minor	D

Conclusion
The overall impact of establishment and spread of NNV was estimated to be **high**.

8.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for NNV in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 6) and found to be:

- Australian ornamental fish—**Very low**.
- Farmed foodfish—**High**.
- Wild fish—**Moderate**.

8.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of NNV for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 7 and found to be:

- Australian ornamental fish—**Negligible**.
- Farmed foodfish—**Very low**.
- Wild fish—**Negligible**.

8.3.7 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 7.

The overall annual risk associated with NNV was found to be **very low**.

Therefore, as the overall annual risk achieves Australia's ALOP, specific biosecurity measures are not considered necessary for this hazard.

9 Salmonid alphavirus

9.1 Background

Salmonid alphavirus (SAV), also known as salmon pancreas disease virus (SPDV), is the aetiological agent of salmon pancreas disease (SPD), pancreas disease (PD) or sleeping disease (SD). For historical reasons, the disease was referred as PD in marine production of *Salmo salar* (Atlantic salmon) and *Oncorhynchus mykiss* (rainbow trout), whereas in freshwater production of rainbow trout, the same pathogenesis was referred to as SD (Graham et al. 2011).

PD was first recorded in farmed Atlantic salmon in Scotland in 1976 (McLoughlin & Graham 2007) and subsequently reported from the United Kingdom and some European countries. The aetiological agents of PD and SD were first isolated in Ireland and France, respectively, and named SPDV (Nelson et al. 1995) and sleeping disease virus (SDV) (Castric et al. 1997). Later, it was concluded that SPDV and SDV were closely related isolates of the same virus species, and the name salmonid alphavirus (SAV) was proposed (Weston et al. 2002).

Infection with SAV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH 2023e) and is listed in Australia's *National list of reportable diseases of aquatic animals* (AHC 2021). Australia has a long history of passive surveillance and a strong system in place to detect incursions, SAV is considered exotic to Australia.

9.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of SAV is warranted.

9.2.1 Agent properties

SAV is an enveloped, spherical, single-stranded, positive-sense RNA virus, approximately 60–70 nm in diameter, with a genome of ~12 kb (Chen et al. 2018). SAV is formally classified by the International Committee on Taxonomy of Viruses (ICTV) as salmon pancreas disease virus (SPDV) in the genus *Alphavirus*, family *Togaviridae* (ICTV 2022). SAV is the most divergent among alphaviruses, with sequence similarity only in parts of the structural and non-structural proteins. This divergence may mean that SAV is ancestral, or it could reflect its adaptation to fish hosts and its lack of evolutionary constraints when compared to other alphaviruses associated with alternating transmission between a mammalian host and arthropod vector (Chen et al. 2018).

SAV has been divided into six genotypes (SAV1–SAV6) based on the nucleic acid sequences for the capsid glycoproteins E2 and the non-structural proteins nsP3 genes (Fringuelli et al. 2008). However, the percentage of nucleotide divergence within genotypes appears to be low (0–4.8% for E2, 0–6.6% for nsP3) (Fringuelli et al. 2008). While SAV1 and SAV2 have been reported to cause disease in freshwater and marine fish, SAV3–SAV6 have only been reported causing disease in marine fish (Weston et al. 2005; WOAH 2023e).

Pathogenesis and infection dynamics had been shown to vary between SAV genotypes (Graham et al. 2011). A cohabitation challenge of Atlantic salmon with fish infected with each of the six SAV genotypes showed that infection with SAV1 and SAV3 resulted in the highest virus loads in heart

tissue as measured by quantitative RT-PCR and in the most extensive histopathological changes while infection with SAV2 and SAV6 showed slower spread, lower virus loads and mild histopathological changes (Graham et al. 2011).

Experiments have suggested that SAV would survive for extended periods in the aquatic environment (Graham et al. 2007b). SAV survival in saltwater and freshwater has been shown to be inversely related to temperature, and to be reduced by the presence of organic matter (Graham et al. 2007b). Experiments conducted with SAV1 when exposed to 4°C, 10°C, 15°C and 20°C showed survival most prolonged at low temperatures and represented by higher half-life (t1/2) values at 4°C when compared to t1/2 values at 20°C (Graham et al. 2007b). SAV was shown to have a half-life of at least 5.7 days at 10°C, 3.6 days at 15°C, and at least 1.5 days at 20°C in seawater. Also, while SAV was detected in seawater 65 days after exposure to 4°C and 10°C, it was not detectable beyond 35 days when exposed to 15°C and beyond 21 days at 20°C (Graham et al. 2007b). At lower temperatures, experiments also showed longer survival times for SAV when in seawater in the presence of organic matter and when compared with freshwater. SAV was shown to have a t1/2 of at least 8.2 days at 10°C in freshwater (Graham et al. 2007b).

In general, alphaviruses can be physically denatured by treating with various chemicals including urea, formaldehyde, beta-propiolactone, detergents, and acids. Infectivity of alphaviruses is also decreased by heat inactivation, exposure to low or high pH and exposure to ultraviolet light (Chen et al. 2018; Graham et al. 2007b). SAV can be rapidly inactivated at 60°C as exposure of SAV1 in liquid media and in the presence of organic matter resulted in no detection of the virus after 1 hour (Graham et al. 2007b).

Host range

SAV is a serious disease agent of farmed Atlantic salmon and rainbow trout. All life stages of Atlantic salmon rainbow trout are susceptible to disease, from smolts to adult fish (Bratland & Nylund 2009; Christie et al. 2007; Graham et al. 2010; McLoughlin, Rowley & Doherty 1998). Different strains of Atlantic salmon may have different susceptibility to SAV. Farmed rainbow trout in both freshwater and saltwater are susceptible to disease at all stages of production (Australian Government Department of Agriculture 2019; WOAH 2023e).

SAV has also been reported affecting other fish species, as summarised in Table 17 and presented in full in Appendix C.

Table 17 Genera susceptible to SAV

Environment	Family	Genus	Permitted import	SAV	
				Susceptible to infection	Positive PCR; no active infection
Marine	Clupeidae	*Clupea*	No	-	N
	Cottidae	*Myoxocephalus*	No	-	+
	Gadidae	*Gadus*	No	-	+
		Melanogrammus	No	-	N
		Merlangius	No	-	N
		Pollachius	No	-	N

Environment	Family	Genus	Permitted import	SAV	
				Susceptible to infection	Positive PCR; no active infection
		Trisopterus	No	-	N
	Labridae	*Labrus*	**Yes**	N[I]	-
	Lepeophtheirus	*Lepeophtheirus*	No	-	N
	Merlucciidae	*Merluccius*	No	-	+
	Pleuronectidae	*Hippoglossoides*	No	N[I]	-
		Limanda	No	-	-
		Platichthys	No	-	N
		Pleuronectes	No	N[I]	-
Euryhaline	Salmonidae	*Oncorhynchus*	n/a	N	N
		Salmo	n/a	N	-
		Salvelinus	n/a	N	-

Permitted import Included on the department's List of permitted live marine ornamental fish suitable for import. **n/a** Not applicable, these species are not included on the department's List of permitted live marine ornamental fish suitable for import and are outside the scope of this review. **N** Susceptible to infection by natural exposure. N[I] incomplete evidence for susceptibility. **E** Susceptible to infection by experimental exposure. **+** Positive PCR but not specified if due to natural or experimental exposure.

Geographical distribution

SAV was initially detected in Scotland and has subsequently been reported in France, Norway, Austria, Germany, Italy, Spain, Scotland, Ireland and England (Fringuelli et al. 2008; Graham et al. 2010; Jansen et al. 2010a; Jansen et al. 2010b; Lewisch et al. 2018; Wallace, McKay & Murray 2017). There is one publication reporting PD in pen-reared Atlantic salmon from the west coast of the United States, but no virus was isolated (Kent & Elston 1987).

Prevalence

Marine ornamental fish

Labrus bergylta (ballan wrasse) is the only confirmed report of SAV infecting a marine ornamental species on the List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A). The report referred to infection of wild ballan wrasse collected in Ireland (Ruane et al. 2018), but no prevalence was estimated.

Salmon

The prevalence rates of infection with SAV are variable. Prevalence of 70–100% have been reported in farmed Atlantic salmon during disease outbreaks in Ireland (Graham et al. 2010). A serological survey for the presence of SAV (SPDV) antibody in farmed Atlantic salmon in Ireland during 1996 reported positive results in 53% of the sites (McLoughlin, Rowley & Doherty 1998). Later, in 2002, an epidemiological survey showed that 59% of Irish sites reported outbreaks of PD (McLoughlin et al. 2003). Surveys during 2003 and 2004 indicated that PD occurred in 62% and 86% of sites, respectively (Ruane et al. 2005).

Fish from 46 freshwater sites in six counties along the Norwegian coastline rearing Atlantic salmon were sampled prior to seawater transfer and followed onto their seawater sites. During the seawater

production phase, fish were sampled three times from 51 seawater sites. SAV was not identified by real-time RT-PCR from smolt tissue samples collected in the freshwater phase. In the seawater phase, SAV3 was detected in 63.9% (n=36) of samples from sites located within the PD endemic region of Norway (south-western coast) but not detected in samples from sites outside the endemic region (n=15). The cumulative incidence of PD during the production cycle amongst sites with SAV detected was 87% (n=23) (Jansen et al. 2010a).

Other fish

SAV has been detected by RT-PCR in some wild marine flatfish species in Scottish waters at prevalence ranging from 0–18%, depending on species and location (Snow et al. 2010). From these, 6.67% (n=30) heart and kidney pools of *Hippoglossoides platessoides* (long rough dab), 9.09% (n=11) of *Pleuronectes platessa* (plaice), and 18% (n=50) of *Limanda limanda* (common dab flatfish) sampled in the vicinity of aquaculture activity tested positive for SAV, while only 4% (n=25) and 2.99% (n=167) individual common dab flatfish caught during two independent sampling trips to areas remote from aquaculture activity tested positive (Snow et al. 2010).

Mortalities

Mortality rates due to SAV have been reported to vary with SAV genotypes, host species, water temperature and time of the year (Bang Jensen et al. 2012; Graham et al. 2011; Graham et al. 2007b). Water temperature is an important environmental variable as it can influence the host immune defence and the pathogen survival (Stene et al. 2014). In cultured fish, SAV has been reported to cause mortalities of over 50% (Australian Government Department of Agriculture 2019; WOAH 2023e). The variability in the mortality rates in cultured fish has been reported to reflect different mitigation and control policies practiced by different aquaculture facilities (Fringuelli et al. 2008). Mortality rates due to infection with SAV in wild fish are largely unknown.

Marine ornamental fish

No reports of mortality events in wild or aquarium wrasse caused by SAV were found.

Salmon

An epidemiological study in Norway, from cages of Atlantic salmon at seawater sites positive for SAV, found that mortality levels following a disease diagnosis varied significantly between populations (range 0.7–26.9%) with the mean percentage of mortality 6.9% (±7.06) (Jansen et al. 2010a). In Ireland, mortalities averaging 12% (range 1–42%) were reported from Atlantic salmon farms suffering from outbreaks of PD in 2002 (McLoughlin et al. 2003). Later, in 2003 and 2004, mean mortalities of 18.8% (range 2–27%) and 14.8% (range 4–35%), respectively, were reported from farms during PD outbreaks (Rodger & Mitchell 2007). Mortalities from 10.9–30% in Atlantic salmon have been reported during outbreaks that occurred in the marine phase of production in two Irish farms (Graham et al. 2010).

Transmission

SAV can be transmitted horizontally by the ingestion of infected tissues and via water (Jansen et al. 2010a; WOAH 2023e). The virus can survive for extended periods in seawater (Graham et al. 2007b). Shedding and transmission of SAV may occur via faeces and mucus, as SAV has been detected persisting in these samples for up to 3 weeks during infection (Graham et al. 2011). Horizontal transmission via inadequately cleaned and disinfected well-boats, or by contaminated equipment or

personnel may also occur (Jansen et al. 2010b). Transmission of SAV from broodstock to progeny is considered unlikely but has not been ruled out (Bratland & Nylund 2009).

Fish that survive disease can become reservoirs of infection (Graham et al. 2010). Natural reservoirs of SAV may include wild fish (Australian Government Department of Agriculture 2019; Snow et al. 2010). Reservoirs of infection may also exist in the environment as SAV outbreaks tend to reoccur in successive fish generations introduced into historically infected sites, despite implementation of management practices including fallowing periods (McLoughlin & Graham 2007; Snow et al. 2010). SAV has been detected by real-time RT-PCR from sea lice *Lepeophtheirus salmonis* infesting Atlantic salmon with PD. Although it was not determined if SAV originated from within the lice, arthropod-borne transmission of SAV has been suggested (Chen et al. 2018; Petterson, Sandberg & Santi 2009).

Mechanism of spread

The mechanism of SAV spread into new countries and/or areas has been attributed to movement of infected live fish (Rodger & Mitchell 2007; Snow et al. 2010). For example, SAV isolates responsible for disease outbreaks in rainbow trout in continental Europe and the United Kingdon are thought to have been introduced in imported fish (Weston et al. 2005)((Snow et al. 2010) citing (Branson 2002)).

Infectious dose

The minimum infectious dose of SAV required to cause disease in susceptible species by experimental challenge or natural infection is not known. However, intraperitoneal injection of Atlantic salmon (mean weight 72 g) with an inoculum containing SAV (SAV1–6, independently) at 5×10^3 $TCID_{50}$/mL resulted in infection. In this study, SAV was able to be transmitted from the injected fish to cohabitant fish, however, the transmission efficiency and the pathological, serological, and virological findings were highly variable between genotypes (Graham et al. 2011). Another experimental study showed that intraperitoneal injection of Atlantic salmon with an inoculum containing SAV at 10^6 $TCID_{50}$/mL, resulted in infection (Desvignes et al. 2002).

9.2.2 Pathogenesis

Outbreaks of PD more often occur in the spring and summer months when the sea temperature is high (McLoughlin & Graham 2007; Rodger & Mitchell 2007). The sea temperature may also have some bearing on the pathogenesis of PD with more acute and shorter-lived outbreaks occurring when temperatures are rising and more chronic outbreaks when temperatures are declining (M. F. McLoughlin, unpublished observations) (McLoughlin & Graham 2007).

Tissue tropism

The heart and the pancreas are the main target organs for infection with SAV. SAV has also been found affecting the brain, kidney, spleen, and gills (Graham et al. 2010; Snow et al. 2010). SAV has been detected in mucous, faeces and serum (Fringuelli et al. 2008; Graham et al. 2010; Graham et al. 2011).

Tissue titre

Tissue screening by real-time RT-PCR of farmed Atlantic salmon during outbreaks of PD showed that the prevalence and persistence of SAV was greatest in gill and heart and lower in kidney and brain (Graham et al. 2010).

The levels of SAV in the serum of infected Atlantic salmon and rainbow trout have been reported to range from 2×10^6–2×10^{11}/mL (Jewhurst et al. 2004). Experimental infection of Atlantic salmon by

cohabitation resulted in detection of SAV RNA (by quantitative RT-PCR) in heart tissue, at 2 weeks post-challenge, of $10^{0.41}$, $10^{0.02}$,$10^{0.24}$ and $10^{0.01}$ copies for SAV1, 3, 5 and 6, respectively (Graham et al. 2011). Comparison of the loads of SAV1, 3, 5 and 6 in heart tissue from 2–8 weeks post-challenge, showed the highest virus loads were from SAV1 and 3, with peak values at 3 weeks post-challenge (values of $10^{0.7}$ and $10^{0.72}$ copies, respectively) (Graham et al. 2011).

Intraperitoneal injection of Atlantic salmon with 10^6 $TCID_{50}$/mL SAV resulted in infection with SAV detectable from day 2 (average viral load 3×10^5 $TCID_{50}$/mL of plasma) and until day 30 post-inoculation (average viral load 10^2 $TCID_{50}$/mL of plasma) (Desvignes et al. 2002). In the study and for the timepoints tested, the levels of SAV peaked at day 4 post-inoculation and progressively decreased through days 9 and 16 post-inoculation, with average viral load of 3×10^6 $TCID_{50}$/mL, 5×10^4 $TCID_{50}$/mL and 8×10^3 $TCID_{50}$/mL, respectively (Desvignes et al. 2002).

9.2.3 Diagnosis

Clinical signs

Clinical signs associated with infection with SAV include yellow mucoid gut contents, petechiae in pyloric fat, pale hearts and/or hemopericardium due to heart rupture, scale pocket oedema, exophthalmos, ascites, and atrophy of red skeletal muscle in chronic cases (Jansen et al. 2017; McLoughlin & Graham 2007). Disease signs on farmed fish include lack of appetite, stunted growth, sluggish swimming and swimming close to the surface and against the current or resting at the bottom of the tank (Australian Government Department of Agriculture 2019; Bang Jensen et al. 2012; Desvignes et al. 2002; Jansen et al. 2017).

Pathology

SAV causes loss and necrosis of the exocrine pancreas, necrosis of cardiomyocytes, myocarditis and skeletal muscle necrosis, degeneration and myositis (Desvignes et al. 2002; Graham et al. 2010; Jansen et al. 2017; McLoughlin & Graham 2007). Additionally, hepatic pyknosis or inflammation consistent with a viral infection and sloughed enterocytes in the caeca have been observed (Graham et al. 2010).

Testing

Chapter 2.3.8 of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH Manual) provides details of the methods currently available for targeted surveillance and diagnosis of SAV (WOAH 2023e). RT-PCR and sequencing are the recommended methods for presumptive and confirmatory diagnosis of SAV. Real-time RT-PCR is the recommended methods for surveillance of apparently healthy animals (WOAH 2023e).

The level of antigenic variation among genotypes is considered low as monoclonal antibodies raised against a specific SAV genotype are likely to cross react with others (Graham et al. 2011; Jewhurst et al. 2004).

9.2.4 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments. DNA-based and cell culture-based virus-inactivated vaccines against SAV are commercially available (Bang Jensen et al. 2012; WOAH 2023e).

9.2.5 Control

Control measures for SAV mainly rely on avoiding exposure to the virus by introducing virus-free fish, coupled with good hygiene practices on farms. Breeding programmes have produced fish with increased resistance to disease caused by SAV, which are now commercially available (WOAH 2023e). Standard disinfection procedures are considered sufficient to prevent contamination of the surface of the egg by SAV (Graham et al. 2007a).

9.2.6 Impact of the disease

Production losses associated with SAV infection are due to mortalities caused during disease outbreaks, reduced growth, increased feed conversion ratio, and reduced quality of infected fish (Jansen et al. 2017). Irish salmon farms presenting high levels of mortality due to SAV during 2003 and 2004, reported a loss of turnover of €35 million and €12 million, respectively (Ruane et al. 2005). A Norwegian study estimated the direct costs of a PD outbreak at a site with 500,000 salmon smolts to be €1.8 million using 2007 figures (Aunsmo et al. 2010). A later study with 2013 figures estimated the direct costs to be €7 million at a site with 1 million smolts (Pettersen et al. 2015).

9.2.7 Current biosecurity measures

There are no current biosecurity measures specific for SAV in marine ornamental fish species.

9.2.8 Conclusion

SAV is present in exporting countries, is not present in Australia and is capable of causing adverse effects. In Australia, infection with SAV is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

9.3 Risk assessment

Based on chapter 2 and the technical information about SAV presented in this chapter, the risk assessment was completed.

A summary of the risk assessment values are shown in Appendix D.

9.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for SAV were that:

- This risk review is generic and therefore the entry assessment assumes that SAV is present in all source countries.
- There is only one report of SAV infecting marine ornamental fish genus on the *Permitted marine species list* (refer Appendix A). Natural infection was reported in wild *Labrus bergylta* (ballan wrasse) collected in Ireland (Ruane et al. 2018). A species of wild-caught wrasse commonly used as cleaner fish, and unlikely to be kept as ornamental fish.
- Prevalence of SAV in marine ornamental fish and wild fish populations is unknown. Prevalence in farmed salmonids can reach 100%.
- SAV can survive in seawater for extended periods, particularly at low temperatures.
- The optimum temperature for SAV isolation and replication is typically 10–15°C (McLoughlin & Graham 2007).

- Pre-export inspection is unlikely to detect marine ornamental fish infected with SAV as the only study reported the infected wrasse showed no clinical signs of disease.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the annual likelihood of entry of SAV was estimated to be **very low**.

9.3.2 Exposure assessment

The key points considered relevant when conducting the direct exposure assessment for SAV were that:

- SAV can be transmitted horizontally by ingestion of infected tissues and via water.
- SAV can survive outside the host for an extended period.
- SAV may be present in sufficient loads in imported fish to cause infection in susceptible species if exposed.
- Salmonid species susceptible to SAV infection are present in Australia.
- SAV typically replicates at temperatures of 10–15°C.
- Because of the holding conditions in aquarium facilities, any ornamental susceptible species grown with, or sharing the same water as infected imported fish, will likely be exposed to viable SAV. However, natural infection has only been detected in wrasse used as cleaner fish which are unlikely to be kept as ornamental fish.
- Farmed foodfish in Australia are known to be susceptible to SAV (e.g. salmonids). However, it is unlikely farmed fish would be in the vicinity of the shore where imported live marine ornamental fish would be released or that they could make their way to a fish cage. Feeding imported marine ornamental fish to farmed foodfish broodstock is assumed to be unlikely because of their high cost and the large volume and specific nutritional profile that farmed foodfish broodstock require.
- Introduction into the wild may occur by direct release of imported marine ornamental fish or their associated waste/water into natural waters. This would be a direct pathway to wild susceptible species. However, the survival of marine ornamental fish released into the wild is expected to be reduced because of their environmental sensitivity.
- SAV has a modest host range present in Australian waters that could be infected. However, wild susceptible species would likely be more scattered compared to the dense populations found in aquaculture, which would reduce opportunities for transmission.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the partial likelihood of exposure of each exposure group to SAV was estimated to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish—**Extremely low**.
- Wild fish —**Very low**.

9.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to SAV was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish— **Extremely low**.
- Wild fish— **Extremely low**.

9.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)

The key points considered relevant when determining the partial likelihood of establishment and spread for SAV were that:

- SAV can be transmitted horizontally by ingestion of infected tissues and via water.
- SAV can survive outside the host for an extended period.
- It is expected that susceptible species in contact with SAV-infected fish would receive an infectious dose.
- Fish that survive SAV infections may become carriers and sources of the virus.
- Salmonid species susceptible to SAV infection are present in Australia.
- SAV typically replicates at temperatures of 10–15°C.
- There is no available treatment for SAV infection.
- Natural infection of SAV in a permitted marine ornamental fish species has currently only been detected in wrasse utilised as cleaner fish, cohoused with foodfish. Whilst stressors associated with aquariums (e.g., stocking density, growing conditions) increases the likelihood of disease establishment, the lack of other susceptible ornamental hosts, and ornamental fish not being cohoused with foodfish, makes it unlikely that SAV will establish in an Australian ornamental fish facility.
- Fish could be moved to other ornamental facilities in Australia. It is expected that SAV could establish in these facilities if present in the fish being translocated.
- SAV is a notifiable fish diseases in Australia including Victoria, New South Wales, Tasmania, Western Australia and South Australia.
- Each state and territory have translocation protocols for aquaculture animals, which typically includes consideration of SAV, which would reduce the likelihood of spread between farmed foodfish populations. Though the movement of ornamental fish between hobbyists and retail premises is difficult to monitor and not necessarily captured under all state and territory legislations.
- If SAV were to establish in ornamental facilities supplying a significant part of the hobby sector or many breeders, it could spread widely within this exposure group.
- It is unlikely that SAV will spread from aquarium industry to farmed foodfish industry due to the lack of exposure pathways and biosecurity practices expected to be in place in facilities which farm susceptible species.

- The likelihood of SAV spread from ornamental facilities to wild populations via escaped fish is unlikely as it is unlikely that live marine ornamental fish would survive in the wild due to their environmental sensitivity, and anticipated propagule pressure.
- Farmed foodfish in Australia are known to be susceptible to SAV (e.g. salmonids).
- If SAV were to establish in farmed foodfish populations it could spread to wild populations through release of water from farms into natural waters. SAV is effectively transmitted through water and can persist in the environment. The spread would be moderated by dilution effects and implementation of biosecurity measures. Spread from cage operated farmed foodfish to wild fish would be even more likely due to the sharing of water.
- SAV spread from foodfish facilities to the aquarium industry is unlikely given the closed systems and lack of exposure pathway between the two exposure groups.
- If one or more index cases of SAV were to occur in the wild, establishment and spread would be less likely than on a farm because the densities of susceptible animals are much less which reduces the opportunities for transmission. However, because SAV can survive in the environment, it could persist until susceptible hosts were to encounter it. The ability of fish to be subclinically infected with SAV and to remain carriers after surviving an infection would also aid its spread.
- If SAV were to establish in the wild, it may spread to cage operated farmed foodfish.
- If SAV were to establish in the wild, especially in waters around aquaculture facilities, it may spread to facilities through water intake. In the absence of effective biosecurity measures, wild infected fish may be transferred into the farms through the inlet water channels.

Conclusion
Based on these considerations and using the descriptors in Table 3, the partial likelihood of establishment and spread of SAV in each exposure group for the outbreak scenario was estimated to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish—**Moderate**.
- Wild fish—**Low**.

Determining adverse impacts resulting from the outbreak scenario
The factors considered relevant when determining the adverse impacts resulting from establishment and spread of SAV included:

Direct effects

The effect on the life or health (including production effects) of susceptible animals and fish

- SAV predominantly infects farmed salmonids and causes over 50% mortality. Production and productivity losses due to SAV would be significant for the Australian salmonid industry with aquaculture production valued at approximately A$1.15 billion in 2021–22 (Tuynman et al. 2023).
- Marine ornamental species other than wrasse may be susceptible to SAV. The marine ornamental industry would be significantly affected by an outbreak of SAV if it caused morbidity and mortality.

- SAV may impact wild fisheries in Australia. There are reports of SAV in wild fish although none were associated with mortalities and declines in catch rates.
- Based on the impacts of SAV infection overseas, SAV establishment and spread in Australia would be expected to cause significant impacts at the national level on the life or health of susceptible species.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There is a moderate host range for SAV in Australia.
- Infection with SAV has been reported in wild fish populations overseas.
- The direct impact of SAV establishment and spread on the living environment is expected to be minor at the district or region level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with SAV is listed as a notifiable disease by WOAH and is included on Australia's *National list of reportable diseases of aquatic animals*. States and territories would be required to report on the occurrence of SAV.
- If SAV was confirmed at a facility or farm, then an attempt at eradication would be undertaken. The cost of an eradication attempt in affected salmonid farms would be significant for the industry.
- To demonstrate that eradication is successful, there would need to be a surveillance exercise over at least two years to confirm freedom, at considerable cost.
- If SAV was confirmed in the environment, the inherent difficulties for the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating SAV from wild populations is unlikely to be undertaken.
- If a movement restriction area were put in place for an outbreak of SAV, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- Eradication and control of SAV is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Movement restriction orders, if put in place, would have indirect impacts on other industries such as seafood suppliers and commercial wild catch fisheries due to the host range of SAV.
- Industries supplying inputs into the affected regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- SAV-infected fish may show clinical signs which would affect their marketability.
- SAV establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Infection with SAV is a WOAH-listed disease. Importing countries may have import requirements for live, fresh, or frozen species susceptible to SAV.
- If SAV were to become established, Australia could use zoning to maintain access to international markets for live susceptible species and non-viable product.
- The impacts of SAV establishment and spread on international trade are likely to be minor at the state or territory level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- SAV has a moderate host range and has been reported in wild fish.
- There are no species listed as endangered in Australia that are related to species known to be susceptible to SAV.
- The impacts of SAV establishment and spread on environmental biodiversity is not expected to be discernible at any level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Fish susceptible to SAV are recreationally fished in Australia and could be affected by mortalities and movement restriction areas put in place which may impact on social amenity.
- In local areas where aquaculture is a major industry, an SAV outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of SAV establishment and spread are expected to be minor at the district or region level.

Table 18 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of SAV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section Determining impacts for detailed methodology).

Table 18 Overall impact of establishment and spread of SAV for the outbreak scenario.

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Significant	F
	The environment (native animals/plants, and non-living environment)	District or region	Minor	C
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	State or territory	Minor	D
	Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernible	A

Effects	Criteria	Level	Impact	Score
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	C

Conclusion

The overall impact of establishment and spread of SAV was estimated to be **high**.

9.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for SAV in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 6) and found to be:

- Australian ornamental fish—**Very low**.
- Farmed foodfish—**High**.
- Wild fish—**Moderate**.

9.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of SAV for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 7 and found to be:

- Australian ornamental fish—**Negligible**.
- Farmed foodfish—**Very low**.
- Wild fish—**Negligible**.

9.3.7 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 7.

The overall annual risk associated with SAV was found to be **very low**. Therefore, as the overall annual risk achieve Australia's ALOP, no specific biosecurity measures are considered necessary for this hazard.

10 Similar damselfish virus and other related ranaviruses

10.1 Background

Ranaviruses comprise a collection of closely related virus strains and variants within the genus *Ranavirus*, family *Iridoviridae* (ICTV 2024). There is significant uncertainty in the taxonomy of isolates within the genus *Ranavirus* (Chinchar et al. 2005). The lineages are constantly changing as additional ranavirus genomes are sequenced and phylogenetic analysis is performed with additional genes or whole genomes (Gray & Chinchar 2015; Owen 2022; Price 2015). Species of ranavirus include the Santee-Cooper ranavirus largemouth bass virus (LMBV, also known as *Ranavirus micropterus1*) (ICTV 2024) and closely related viral isolates as Similar damselfish ranavirus (SRDV), koi ranavirus (KIRV), and other highly similar viruses.

SRDV was first reported causing mortality in farmed *Pomacentrus similis* (similar damselfish) in South India in 2017 (Sivasankar et al. 2017b). KIRV was isolated from mortality events of farmed *Cyprinus carpio* (koi) in South India in 2015 (George et al. 2015). Natural infection with SRDV or KIRV has not been reported beyond the region of initial detection.

SRDV is the only ranavirus that complies with the criteria described in the World Organisation for Animal Health *Aquatic animal health code* (WOAH Code) Article 2.1.2 *Hazard Identification* (WOAH 2023a) and has been retained as hazard. As SRDV, KIRV and LMBV are closely related virus isolates, KIRV and LMBV are also considered in this chapter.

Infection with *Ranavirus* is listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (WOAH 2023a). On Australia's *National list of reportable disease of aquatic animals*, infection with Singapore grouper iridovirus is included for finfish species, and infection with *Ranavirus* species is included for amphibians (AHC 2021). Australia has a long history of passive surveillance and a strong system in place to detect incursions. SRDV, KIRV and LMBV are considered exotic to Australia.

10.2 Technical information

10.2.1 Agent properties

Ranaviruses are large icosahedral, double-stranded DNA (dsDNA) containing viruses. Viruses within the genus *Ranavirus* belong to the subfamily *Alphairidovirinae* and family *Iridoviridae* (ICTV 2024). The International Committee of Taxonomy and Viruses (ICTV) has formally classified 7 virus species as members of the genus Ranavirus (ICTV 2024). These include *Ranavirus epinephelus1* (SGIV), *Ranavirus alytes1* (common midwife toad virus), *Ranavirus ambystoma1* (Ambystoma tigrinum virus), *Ranavirus gadus1* (lumpfish ranavirus), *Ranavirus micropterus1* (largemouth bass virus), *Ranavirus perca1* (Epizootic haematopoietic necrosis virus) and *Ranavirus rana1* (Frog virus 3, FV3) (ICTV 2024).

The ICTV outlines criteria for characterising and distinguishing ranavirus isolates. Criteria include amino acid and nucleotide sequence identity, phylogeny, principal host species, genome size, genetic co-linearity, and gene content. Within *Ranavirus*, many isolates show >90% similarity in sequence

identities for the major capsid protein and other conserved points. Consequently, the ICTV considers ranavirus isolates as members of the same viral species if they share >95% amino acid identity based on a concatenated set of 26 core iridovirid genes, and display phylogenetic relatedness, a co-linear gene arrangement, similar genomic size, and similar G+C content (ICTV 2023a).

LMBV, the first Santee-Cooper ranavirus, was identified in *Micropterus salmoides* (largemouth bass) from Santee-Cooper reservoir, and later reported from other river systems in the United States (Whittington, Becker & Dennis 2010). LMBV is formally classified by the ICTV as species *Ranavirus micropterus1* (ICTV 2024), which include the virus isolates, doctor fish virus (DFV) and guppy virus 6 (GV-6) (Chinchar et al. 2017a; ICTV 2023a, b, 2024). Genetic and phenotypic differences have been reported among LMBV isolates (Goldberg et al. 2003). In addition, other two virus have been reported to be closely related to LMBV, the similar damselfish ranavirus (SRDV) and koi ranavirus (KIRV), isolated from farmed *Pomacentrus similis* (similar damselfish) (Sivasankar et al. 2017b) and *Cyprinus carpio* (koi carp) (George et al. 2015), respectively. Sequence analysis of the major capsid protein (MCP) of LMBV and KIRVs showed a 99.9% similarity (George et al. 2015). SRDV showed 99.82% identity with LMBV and 99.29% identity with KIRV across a 1130 fragment of the MCP (Sivasankar et al. 2017b). Serological studies have also shown that SRDV and KIRV share similar immunogenic epitopes on their capsid proteins and antigenic similarity (Sivasankar et al. 2017b).

At the time of preparing this report, SRDV is the only exotic *Ranavirus* confirmed to infect live marine ornamental fish currently included on the department's List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*). In experimental studies, KIRV was found to grow on *Amphiprion sebae* (sebae clownfish) cell lines (George et al. 2015). As SRDV is the only *Ranavirus* that has been shown to infect a live permitted species of marine ornamental fish, it will be considered the primary risk, with KIRV and LMBV being considered as they are closely related viral isolates.

In general, members of *Ranavirus* loose infectivity rapidly at pH 2.0–3.0 and at temperatures above 50°C (ICTV 2023a). Virions are sensitive to ether, chloroform, sodium deoxychlorate and phospholipase A (ICTV 2023a). LMBV survives freezing as it has been reported to remain viable in fish tissues frozen at - 10°C for at least 155 days (Plumb & Zilberg 1999b).

10.2.2 Epidemiology

Host range

Species which are susceptible to infection with SRDV, KIRV and LMBV, is limited, as summarised in Table 19, and presented in full in Appendix C.

Table 19 Genera susceptible to SRDV, KIRV and LMBV

Environment	Family	Genus	Permitted import	Susceptible to infection with SRDV	Susceptible to infection with LMBV	Susceptible to infection with KIRV
Marine	Pomacentridae	*Pomacentrus*	Yes	N	-	-
Euryhaline	*Latidae*	*Lates*	n/a	E	E	-
	Cichlidae	*Oreochromis*	n/a	-	-	E
Freshwater	Cyprinidae	*Cyprinus*	n/a	E	-	N
		Carassius	n/a	-	-	E

Environment	Family	Genus	Permitted import	Susceptible to infection with SRDV	Susceptible to infection with LMBV	Susceptible to infection with KIRV
		Labeo	n/a	-	-	E
		Necomis	n/a	-	E	-
	Centrarchidae	*Micropterus*	n/a	-	N	-
		Lepomis	n/a	-	E	-
		Ambloplites	n/a	-	E	-
		Poxomis	n/a	-	E	-
	Moronidae	*Scortum*	n/a	-	N	-
		Morone	n/a	-	N, E	-
	Esocidae	*Esox*	n/a	-	E	-
	Sciaenidae	*Aplodinotus*	n/a	-	E	-
	Castostomidae	*Minytrema*	n/a	-	E	-
	Channidae	*Channa*	n/a	-	E	-

Permitted import Included on the department's List of permitted live marine ornamental fish suitable for import. **n/a** Not applicable because freshwater species. **N** Susceptible to infection by natural exposure. **E** Susceptible to infection by experimental exposure.

SRDV and KIRV have been reported to affect juvenile to adults, with juveniles being more susceptible. LMBV is most prevalent in young to intermediate aged fish (1.5 years to 6 years), with reduced transmissions amongst fingerlings, and assumed increased mortality in adults (Maceina & Grizzle 2006).

Although SRDV has only been reported causing natural infection and mortality in ornamental facilities and farms holding similar damselfish (Sivasankar et al. 2017b), it has experimentally infected *Lates calcarifer* (barramundi) fingerlings following challenge by intraperitoneal injection (Riji John, Sivasankar & George 2023) and freshwater fish *Channa striata* (snakehead murrel) cell lines (Sivasankar et al. 2017b).

KIRV has only been reported causing natural infection and mortality in koi carp, but experimental challenge has expanded the possible host range. The cell lines of snakehead murrel, barramundi, sebae clownfish, *Lepomis macrochirus* (bluegill), *Pimephales promelas* (fathead minnow, *Epithelioma papulosum cyprini* cell line), and *Ictalurus nebulosis* (brown bullhead) have all shown KIRV growth once exposed (George et al. 2015). Experimental challenge by intraperitoneal injection of KIRV produced pathogen-specific PCR results in *Carassius auratus* (goldfish), *Oreochromis niloticus* (Nile tilapia), *Cyprinus carpio* (common carp) and *Labeo rohita* (rohu) at 3 days post injection. Although clinical signs or mortalities were not observed in the challenged fish, histological lesions were present in some tissues (Kaviarasu et al. 2022; Kaviarasu et al. 2020).

Geographical distribution

SRDV and KIRV were both first reported in South Indian ornamental fish breeding and rearing farms (George et al. 2015; Sivasankar 2017). No reports of either virus distributing beyond this region, and these populations, were found.

LMBV was first reported to cause mortality in the early 1990s, in wild populations of largemouth bass in the United States (Grizzle et al. 2002). The initial reports were isolated to Florida and South Carolina (Grizzle et al. 2002), but by 2003 LMBV had caused clinical signs and mortality events in wild and cultured populations of largemouth bass in over 20 states (Zhao et al. 2023b). First reports of LMBV in China occurred in 2006-2008, when cultured populations across several farms in the Guangdong Province experienced ulcerative clinical signs (Zhao et al. 2023b).

Prevalence

Data on the prevalence of ranavirus infections in live marine ornamental fish is scarce, likely due to the high mortality and quick progression to death associated with ranaviruses.

Marine ornamental fish

SRDV has only been reported in farmed *Pomacentrus similis* but no prevalence estimates were given. KIRV and LMBV have not been reported in marine ornamental fish species.

Other fish

No reports of the prevalence of KIRV were found. There are few published surveys of LMBV in wild populations of fish. A survey of 2,876 adult largemouth bass collected in 2000 from 14 reservoirs and 8 river basins in Texas, reported an overall average prevalence of 1.5%. Prevalence of LMBV in infected water sources averaged 5%, but ranged from 1.7% to 13.3% (Southard, Fries & Terre 2009). In 2003, adult largemouth bass in Mississippi were reported to have LMBV prevalence of about 40% at Lake Monroe, and of about 25.8% at Tombigee State Park Lake (Schramm Jr & Davis 2006). LMBV was detected in 85% (n = 60) of wild largemouth bass in pool 8 of the upper Mississippi river, in 2016 (Leis et al. 2018). Surveys conducted in China across 2019 – 2021 of largemouth bass in the Guangdong region, and surrounding areas, detected LMBV at a prevalence of 63.62% (n = 723) (Zhao et al. 2023a).

Mortalities

Santee-Cooper viruses are reported to show high virulence variations in different species of fish (Sivasankar 2017; Whittington, Becker & Dennis 2010), therefore mortality rates vary by fish species. It has been suggested that morbidity and mortality due to LMBV in wild populations of largemouth bass could be a result of seasonal environmental factors, specifically water temperature (Goldberg et al. 2003; Plumb & Zilberg 1999b; Whittington, Becker & Dennis 2010). Heavy mortalities have been reported for SRDV in natural and experimental infections. Mortality of hosts injected with either KIRV or LMBV have produced varied results dependant on species and temperature (Boonthai et al. 2018; Kaviarasu et al. 2022).

Marine ornamental fish

Significant mortalities are reported to occur in captive bred similar damselfish due to SRDV (Sivasankar et al. 2017b). Experimentally, intraperitoneal injection of SRDV in live fingerlings of similar damselfish caused 93.33% mortality of the population (Sivasankar et al. 2017b).

Other fish

Under experimental conditions, cumulative mortalities of 68.75% were reported in live barramundi fingerlings following intraperitoneal injection with SRDV (Sivasankar et al. 2017b). Although no histopathological investigations were done on the fish.

KIRV is known to cause heavy mortalities (90-100%) in the original host species, koi carp (George et al. 2015). However, when tested against other live fish species (goldfish and tilapia), positive infection did not result in clinical signs or mortalities (Kaviarasu et al. 2022). There are no other reports of KIRV causing mortalities.

LMBV has been observed to cause mortalities in largemouth bass from wild populations in Northern America, to cultured populations in China (Zhao et al. 2023b). LMBV has been observed to cause heavy mortality (up to 90 – 100%) both naturally and experimentally in largemouth bass (Fu et al. 2022; Maceina & Grizzle 2006). Whilst juveniles are reported to have the highest prevalence of LMBV, it is assumed that adults are more susceptible to rapid mortality, with reports of heavy mortality being found in wild adult populations (Maceina & Grizzle 2006). LMBV was surveyed for in China across 2016 to 2018 in largemouth bass, the cumulative mortality of disease outbreaks was estimated to be 40-55% when the water temperature was 22-28°C (Zhao et al. 2023b). Experimentally, intraperitoneal injection of LMBV in juvenile largemouth bass resulted in overall mortality of 60% within 4 days (Plumb & Zilberg 1999b) and up to 100% mortalities by 5 days (Plumb & Zilberg 1999a). Mortalities of 17% and 52% had been reported in largemouth bass following experimental infection by cohabitation with infected fish (Plumb et al. 1999; Plumb & Zilberg 1999a). Mortalities of 63% and 10% have also been reported in juvenile striped bass *Morone saxatilis* following experimental infection by intraperitoneal injection and immersion, respectively (Plumb & Zilberg 1999a).

Transmission

Given the known outbreaks of SRDV in captive-bred populations, the natural mode of transmissions is likely to be horizontal through direct contact with virus contaminated water, or via cohabitation with infected fish. Experimentally, infection has only been tested using intraperitoneal injections (Riji John, Sivasankar & George 2023; Sivasankar et al. 2017a). SRDV shed into the water from infected fish, and the transmission of SRDV from broodstock to progeny, has not been investigated.

SRDV has been shown to infect live marine and brackish hosts, and the cell lines of freshwater hosts (Sivasankar et al. 2017b). Study has not been conducted to determine if these hosts can further transmit SRDV across freshwater and marine environments using euryhaline species.

The natural mode of transmission for KIRV has been shown to be horizontally, and through contaminated water (George et al. 2015). Like SRDV, KIRV has been shown to infect marine, brackish, and freshwater hosts through live, and cell line, experimentation (George et al. 2015). The transmission of KIRV from broodstock to progeny has not been investigated.

The natural mode of transmission of LMBV may be primarily horizontal through direct contact with virus contaminated water or via cohabitation with infected fish (Woodland, Noyes & Grizzle 2002). Experimentally, horizontal transmission via cohabitation, water, and ingestion of infected fish has been achieved (Beck et al. 2006; Woodland et al. 2002). Transmission of live virus via shedding into the water from infected fish is possible (Beck et al. 2006). Fish which survive infection with LMBV have the potential to act as carriers (Zhao et al. 2020). Transmission of LMBV from broodstock to progeny is suspected (Woodland, Noyes & Grizzle 2002). LMBV has been shown to infect freshwater, brackish, and marine fish hosts.

Mechanism of spread

The mechanism of spread of ranaviruses into new countries and/or areas has been attributed to the international trade of live fish, amphibians and reptiles, that are sold commercially as food or as ornamental and pet species (Herath, Ellepola & Meegaskumbura 2021; Miller, Gray & Storfer 2011; Whittington, Becker & Dennis 2010).

No reports of SRDV or KIRV outside the South Indian region were found.

Initial reports of LMBV were isolated from wild populations of largemouth bass in North America in the early 1990's (Grizzle et al. 2002; Plumb et al. 1996). LMBV was later reported in China (Zhao et al. 2023b; Zhao et al. 2020). Although the mechanism of spread of LMBV into new regions has not been determined, the introduction of largemouth bass for aquaculture is thought to have increased the dispersal of ranaviral infections in Asia (Herath, Ellepola & Meegaskumbura 2021), including infections by Santee Cooper ranaviruses (Plumb et al. 1996).

Infectious dose

The minimum infectious dose of SRDV required to cause disease in susceptible species by experimental challenge or natural infection is unknown. However, intraperitoneal injection in barramundi fingerlings at 50 µl per fish, or 50 µl of 10^{-2} diluted virus, caused a cumulative mortality of 62.5%, and 68.75%, 35 days post-inoculation, respectively (Sivasankar et al. 2017b). Intraperitoneal injection of a saline solution containing $10^{6.8}$ $TCID_{50}$ of LMBV infected more than 90% of exposed fish (the titre of infected tissue was up to $10^{8.8}$ $TCID_{50}$/g), resulting in up to 80% mortality at 4 days post infection (Plumb & Zilberg 1999a).

10.2.3 Pathogenesis

Santee Cooper viruses are reported to show high virulence variations in different species of fish (Sivasankar 2017; Whittington, Becker & Dennis 2010). Clinical variability has also been reported as a notable feature of LMBV infection (Goldberg et al. 2003). Outbreaks attributed to LMBV have mostly occurred during summer to autumn, and it has been reported that LMBV is prevalent when the water temperature is between 25°C and 30°C (Goldberg et al. 2003; Grant et al. 2003; Zhao et al. 2023b).

Tissue tropism

In the similar damselfish, infection with SRDV has primarily been observed in the spleen, kidney, and liver (Sivasankar et al. 2017b). In foodfish, barramundi and largemouth bass, infection with SRDV, or LMBV, has been observed in spleen, kidney, liver, gill, heart, brain and intestines (Beck et al. 2006; Kaviarasu et al. 2022; Sivasankar 2017).

Tissue titre

Concentration of SRDV or KIRV in naturally infected fish has not been estimated. In experimentally infected marine and freshwater cell lines, concentration of SRDV was relatively similar, ranging between 10^5 and 10^6 $TCID_{50}$/mL (Sivasankar et al. 2017b). In experimentally infected snakehead murrel cell-lines, KIRV titre had a concentration of $10^{8.5 +/- 0.94}$ $TCID_{50}$/mL (George et al. 2015). LMBV concentrations of $10^{2.6-7.8}$ $TCID_{50}$/g of fish tissue have been reported in naturally infected largemouth bass (Plumb et al. 1999). Woodland et al. (2002) reported virus titres of $10^{2.8- 9.5}$ $TCID_{50}$/g (the average weight of the fish was 64g) in largemouth bass orally infected with inoculum of $10^{4.6}$ to $10^{6.1}$ $TCID_{50}$/mL.

10.2.4 Diagnosis

Clinical signs

Clinical disease of SRDV is often acute and can affect a high proportion of the population, resulting in mass mortality (Sivasankar et al. 2017b). Naturally, and experimentally, infected similar damselfish typically present with sudden jerky movements, abnormal swimming, lethargy, anorexia, discolouration of skin, settling and the bottom of tanks, and haemorrhagic lesions (Riji John, Sivasankar & George 2023).

Clinical disease of KIRV, as experienced by cultured Koi in South Indian breeding farms, is often acute and can affect a high proportion of the population, resulting in mass mortality (George et al. 2015). Naturally infected Koi experienced clinical signs which include jerky movements, abnormal swimming, lethargy, discolouration, and eventually mortality (Riji John, Sivasankar & George 2023). Experimentally infected hosts have had varied responses to positive infection, with some not developing any clinical signs (Kaviarasu et al. 2022).

Clinical disease of LMBV can often be acute, affect a high proportion of the population, and result in mass mortality (Zhao et al. 2023b). Infection with LMBV has been shown to cause clinical signs which include scale drop, lesions, lethargy, abnormal swimming, and mortality (Woodland et al. 2002). Like KIRV, experimental settings have not always been able to reproduce these signs (Plumb et al. 1996).

Pathology

There are no reports concerning the pathology of SRDV in infected fish.

Following experimental infection with KIRV, histopathological study revealed focal necrosis, shrunken glomerulus and detachment of the epithelium of tubules in kidney tissue of goldfish. Similarly experimentally infected tilapia, common carp and rohu presented focal necrosis of glomerulus, and detachment of the epithelium of tubules in kidney tissue (Kaviarasu et al. 2022; Kaviarasu et al. 2020). Melanomacrophage centres have also been reported in the spleen of experimentally infected rohu (Kaviarasu et al. 2020).

Pathological changes caused by LMBV include hepatocytes volume shrinkage, nucleus pyknosis, necrosis and cell membrane disintegration. Other observed changes include loss of cellular outlines, spleen necrosis, and focal cellular necrosis (Xu et al. 2010). Juvenile fish infected with LMBV have shown acute haemorrhages and visceral enlargement, and viral growth in the liver, spleen, kidney, gills, and intestinal tissue. Histopathological analysis of infected juveniles also showed infiltration of the inflammatory cell and histiocyte necrosis (Liu et al. 2023)

Testing

Chapter 2.1.3 of the WOAH Manual of diagnostic tests for aquatic animals (OIE 2021d) provides details of the methods currently available for surveillance and confirmatory diagnosis of ranavirus in amphibians. Cell culture, antigen-capture enzyme linked immunosorbent assay (ELISA) and PCR restriction endonuclease analysis are recommended for targeted surveillance of all amphibian stages. Cell culture, PCR-restriction endonuclease analysis and PCR sequence analysis are the recommended methods for confirmatory diagnosis (OIE 2021d).

PCR and real time PCR designed to detect SRDV, KIRV and LMBV are available (George et al. 2015; Sivasankar et al. 2017a; Sivasankar et al. 2023).

10.2.5 Treatment

There are no scientifically confirmed reports of effective chemotherapy or immunostimulation treatments. An inactivated SRDV vaccine has been developed (Sivasankar et al. 2017a) but is not commercially available.

10.2.6 Control and prevention

General control measures to prevent infections in ornamental facilities can include screening of individuals as ranavirus negative before movement, isolation of positive individuals, culling, minimising possible stressors, use of filtered water, maintenance of low host densities, adhering to biosecurity procedures to prevent cross contamination and disinfection of animal enclosures, footwear and equipment (Gray & Chinchar 2015; Miller, Gray & Storfer 2011; OIE 2021b)

10.2.7 Impact of the disease

SRDV and KIRV can cause high levels of morbidity and mortality among fish species in marine, brackish and freshwater (George et al. ; Kaviarasu et al. 2022; Sivasankar 2017). SRDV and LMBV have both been shown to cause mortality in commercially important fish species, barramundi, and largemouth bass, respectively (Sivasankar et al. 2023). Outbreaks of LMBV in China, in cultured populations of largemouth bass are considered the greatest economic threat of the production (Zhao et al. 2023b).

10.2.8 Current biosecurity measures

There are no current biosecurity measures specific for SRDV, KIRV or LMBV in marine ornamental fish species.

10.2.9 Conclusion

SRDV, KIRV or LMBV, are present in exporting countries, are not present in Australia and are capable of causing adverse effects. In Australia, infection with ranavirus is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

10.3 Risk assessment

Based on chapter 2 and the technical information about SRDV, KIRV and LMBV presented in this chapter, the risk assessment was completed.

A summary of the risk assessment values are shown in Appendix D.

10.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for SRDV, KIRV and LMBV, were that:

- The risk review is generic and therefore the entry assessment assumes that SRDV, KIRV and LMBV are present in all source countries.
- There is only one report of SRDV infecting marine ornamental fish on the *Permitted marine species list* (refer Appendix A). Natural infection was reported in captive breed similar damselfish (genus *Pomacentrus*) in India (Sivasankar et al. 2017b).
- Prevalence of SRDV, or KIRV, in infected populations has never been estimated. Prevalence for LMBV has been observed as low as 1.3% to as high as 85% in North American populations of

largemouth bass (Leis et al. 2018; Southard, Fries & Terre 2009), and as high as 63.62% in Chinese largemouth bass populations (Zhao et al. 2023b).

- The viral load of SRDV in infected imported fish would likely be sufficient to cause infection in susceptible species.
- Whilst studies have not been conducted on the survivability of SRDV, KIRV or LMBV in water, other ranaviruses have been reported to survive in water for extended periods (Munro et al. 2016; WOAH 2023c).
- Pre-export inspection may detect marine ornamental fish showing clinical signs that are typical of infection with SRDV, and remove them before export. Live marine ornamental fish with mild, or no clinical signs, would not be identified through visual inspection.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the annual likelihood of entry of SRDV was estimated to be **very low**.

10.3.2 Exposure assessment

The key points considered relevant when conducting the direct exposure assessment for SRDV, and similar viral species, KIRV or LMBV were that:

- SRDV, KIRV and LMBV can be transmitted horizontally by ingestion of infected tissues and via water.
- SRDV, KIRV and LMBV may survive in water outside the host for an extended period, as other ranaviruses.
- SRDV, KIRV and LMBV may be present in sufficient loads in imported fish to cause infection in susceptible species if exposed.
- Species susceptible to SRDV, KIRV and LMBV infection are present in Australia, including the ornamental fish similar damselfish, barramundi and the koi and common carp.
- Ranaviruses often cause disease in warmer temperatures and remain viable in winter. LMBV has been shown to be most impactful in largemouth bass populations in temperatures ranging from 25-30°C, but has caused 50% morality in temperatures as low as 22°C (Zhao et al. 2020).
- Because of the holding conditions in aquarium facilities, any ornamental susceptible species. grown with, or sharing the same water as infected imported fish, will likely be exposed to viable SRDV, KIRV or LMBV. However, natural infection has only been detected in the marine ornamental similar damselfish. Pathogen-specific PCR results for infection with KIRV have been reported in the freshwater ornamental goldfish following experimental infection (Kaviarasu et al. 2022), however an active infection had not been demonstrated.
- Farmed foodfish in Australia are known to be susceptible to SRDV and KIRV (i.e. barramundi). However, it is unlikely farmed fish would be in the vicinity of the shore where imported live marine ornamental fish would be released or that they could make their way to a fish cage. Feeding imported marine ornamental fish to farmed foodfish broodstock is assumed to be unlikely because of their high cost and the large volume and specific nutritional profile that farmed foodfish broodstock require.

- Introduction into the wild may occur by direct release of imported marine ornamental fish or their associated waste/water into natural waters. This would be a direct pathway to wild susceptible species. However, the survival of marine ornamental fish released into the wild is expected to be reduced because of their environmental sensitivity.
- SRDV, KIRV or LMBV have a limited host range present in Australian waters that could be infected. However, wild susceptible species would likely be more scattered compared to the dense populations found in aquaculture, which would reduce opportunities for transmission.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the partial likelihood of exposure of each exposure group to SRDV, KIRV and LMBV was estimated to be:

- Australian ornamental fish – **Very low**.
- Farmed foodfish – **Extremely low**.
- Wild fish – **Very low**.

10.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each group to SRDV, KIRV and LMBV was determined by combining the likelihood of entry and the partial likelihood of exposure using the mature in Figure 3, and was found to be:

- Australian ornamental fish – **Extremely low**.
- Farmed foodfish – **Extremely low**.
- Wild fish – **Extremely low**.

10.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)
The key points considered relevant when determining the partial likelihood of establishment and spread for SRDV, KIRV or LMBV were that:

- KIRV and LMBV can both transmit horizontally via contact between fish and through water. Given the outbreaks of SRDV in breeding ponds, the virus likely transmits similarly. LMBV is also known to transmit horizontally through ingestion of infected fish (Beck et al. 2006; Woodland et al. 2002) and potentially transmit vertically (Woodland, Noyes & Grizzle 2002).
- SRDV, KIRV and LMBV may survive in water outside the host for an extended period, as other ranaviruses.
- It is expected that susceptible species in contact with SRDV-infected fish would be exposed to virus loads sufficient to cause infection.
- Whilst information on the transmission of SRDV is limited, infections of LMBV have shown that fish who survive can become carriers and sources of the virus (Zhao et al. 2020).
- Species susceptible to SRDV, KIRV and LMBV infection are present in Australian waters, including the ornamental fish similar damselfish, barramundi and the koi and common carp. At the time of preparing this report, natural infections by SRDV and KIRV have only been reported in similar damselfish and koi carp. Experimental infection by injection has been reported in barramundi and common carp but no studies using a natural exposure route were found.

- Ranaviruses often cause disease in warmer temperatures and remain viable in winter over colder seasons. LMBV has been shown to be most impactful in largemouth bass populations in temperatures ranging from 25-30°C, but has caused 50% morality in temperatures as low as 22°C (Zhao et al. 2020).
- There is no available treatment for SRDV, KIRV and LMBV infection.
- Natural infection with SRDV, KIRV or LMBV in a permitted marine ornamental fish species has currently only been detected in the similar damselfish. Whilst stressors associated with aquariums (e.g., stocking density, growing conditions) increases the likelihood of disease establishment, the lack of other susceptible ornamental hosts, and ornamental fish not being cohoused with foodfish, makes it unlikely that SRDV, KIRV or LMBV will establish in an Australian ornamental fish facility.
- Fish could be moved to other ornamental facilities in Australia. It is expected that SRDV, KIRV or LMBV could establish in facilities holding susceptible species if present in the fish being translocated. However, this establishment would be moderated by the narrow host range of SRDV, KIRV or LMBV.
- Each state and territory have translocation protocols for aquaculture animals but may not include consideration of ranaviruses including SRDV, KIRV or LMBV. The movement of ornamental fish between hobbyists and retail premises is difficult to monitor and not necessarily captured under all state and territory legislations.
- Farmed foodfish in Australia (i.e. barramundi) have been reported to be susceptible to SRDV following experimental infection by injection.
- It is unlikely that SRDV, KIRV or LMBV will spread from Australian ornamental fish to farmed foodfish due to the narrow host range, the lack of exposure pathways and biosecurity practices expected to be in place in facilities which farm susceptible species.
- It is unlikely that SRDV, KIRV or LMBV will spread from Australian ornamental facilities to wild populations via escaped or released fish, given the narrow host range, their environmental sensitivity and the anticipated propagule pressure required to ensure establishment of a population.
- If SRDV, KIRV or LMBV were to establish in farmed foodfish populations (i.e. barramundi), it could spread to wild populations through release of water from farms into natural waters. SRDV, KIRV or LMBV may remain viable in water for months. The spread would be moderated by dilution effects and implementation of biosecurity measures. Spread from cage operated farmed foodfish to wild fish would be even more likely due to the sharing of water.
- Spread of SRDV, KIRV or LMBV from foodfish facilities to Australian ornamental fish is unlikely given the closed systems, lack of exposure pathway between the two exposure groups, and the limited marine ornamental fish susceptible to SRDV, KIRV or LMBV.
- KIRV has been reported causing natural infection and heavy mortalities in koi carp (George et al. 2015). In common carp, experimental infection by injection with KIRV resulted in histopathological lesions but no mortality (Kaviarasu et al. 2020). There is a significant wild population of feral carp that may become infected. Carp dominate freshwater fish communities in south-eastern Australia. In many areas they comprise a significant proportion of fish biomass,

sometimes exceeding 80% or 350 kg/ha in parts of the Murray-Darling Basin (Australian Government & FRDC 2022).

- If one or more index cases of SRDV, KIRV or LMBV were to occur in the wild, establishment and spread would be more likely than in Australian ornamental fish populations due to a wider host range being available (e.g. barramundi, koi and common carp) and its effective transmission via water. The ability of fish to be subclinically infected with SRDV, KIRV and LMBV, and to remain carriers after surviving an infection would also aid spread.
- If SRDV, KIRV or LMBV were to establish in the wild, it may easily spread to cage operated farmed foodfish due to the sharing of water and opportunities for direct fish contact.
- If SRDV, KIRV or LMBV were to establish in the wild, especially in waters around foodfish aquaculture facilities, it may spread to facilities through water intake due to these viruses being transmissible through water. The spread may be moderated through water dilution, biosecurity measures in place and the narrow host range.
- Spread of SRDV from the wild to the Australian ornamental fish population may occur in cases of subclinically affected species being collected from the wild for sale by the aquarium industry, but the host range is very narrow (i.e. Similar damselfish) and any clinically affected fish are unlikely to be harvested from the wild.

Conclusion
Based on these considerations, and using the descriptors in Table 3, the partial likelihood of establishment and spread of SRDV each exposure group for the outbreak scenario was estimated to be:

- Australian ornamental fish – **Extremely low**.
- Farmed foodfish – **Low**.
- Wild fish – **Low**.

Determining adverse impacts resulting from the outbreak scenario
The factors considered relevant when determining the adverse impacts in establishment and spread of SRDV:

Direct effects

The effect on the life or health (including production effects) of susceptible animals and fish

- SRDV and KIRV can cause high mortalities in farmed foodfish, barramundi. Production and productivity losses due to SRDV or KIRV would be significant for the Australian barramundi industry with aquaculture production valued at approximately A$60 million (Tuynman et al. 2023). This value is likely an underestimate due to some data not being available at the time of the report's release.
- SRDV has been observed to cause mortality in infected marine ornamental fish. Losses due to SRDV could be anticipated to occur in, and impact, the marine ornamental industry.
- KIRV has been reported causing natural infection and heavy mortalities (90-100%) in koi carp (George et al. 2015). The domestic koi carp industry, estimated to conservatively expend $20–52 million Australia-wide (DAFF 2022), would be significantly affected by an outbreak of KIRV.

- SRDV and KIRV may impact wild barramnudi fisheries in Australia. There are reports of LMBV in wild largemouth overseas associated with mortalities and declines in catch rate (Grizzle et al. 2002; Plumb et al. 1996).
- Based on the impacts of SRDV, KIRV and LMBV, their establishment and spread in Australia would be expected to cause minor impacts at the national level on the life or health of susceptible species.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- SRDV, KIRV and LMBV can infect wild fish present in Australia.
- There are reports of infection, either subclinical or associated with mortalities, in wild fish populations overseas.
- KIRV has been reported causing natural infection and heavy mortalities (90–100%) in koi carp (George et al. 2015). Experimental infection by injection with no mortalities has been reported in common carp (Kaviarasu et al. 2020). Common carp are considered one of the worst introduced pest species in Australia, the national biomass of common carp ranges from 200,000 tonnes to possibly up to approximately 1 million tonnes under ideal breeding conditions (Australian Government & FRDC 2022). If KIRV were to cause mortalities in the wild carp population following natural infection, this could result in a significant biomass that could affect water quality and cause native fish kills if not effectively removed and disposed of.
- The direct impact or SRDV, KIRV and LMBV, establishment and spread on the living environment is expected to be minor at the national level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- States and territories would be required to report the occurrence of exotic ranavirus species.
- If SRDV, KIRV or LMBV was confined to an ornamental facility, or foodfish farm, then an attempt at eradication may be undertaken. The cost of an eradication attempt in affected foodfish farms would be significant to the industry.
- To demonstrate the eradication is successful, there would need to be a surveillance exercise over at least two years to confirm freedom, at considerable cost.
- If a movement restricted were to be put in place for an exotic ranavirus, there would ongoing costs associated with the surveillance, monitoring and implementation of the area.
- If infection with SRDV, KIRV or LMBV was confirmed in the environment, the inherent difficulties for eradication of aquatic diseases from wild populations would mean that a campaign aimed at eradicating the new ranavirus from wild populations is unlikely to be undertaken.
- KIRV has been reported to cause natural infection and heavy mortalities (90–100%) in koi carp (George et al. 2015). Experimental infection by injection has been reported in common carp, but there were no mortalities (Kaviarasu et al. 2020). In many areas of south-eastern Australia common carp comprise a significant proportion of fish biomass, sometimes exceeding 80% or 350 kg/ha in parts of the Murray-Darling Basin (Australian Government & FRDC 2022). The biomass of carp in south-eastern Australia was estimated to be 205,774 tonnes (Stuart et al.

2021). If KIRV were to cause mortalities in the wild carp, an outbreak of KIRV could result in a significant biomass that would be required to be effectively cleaned-up (Silva, Bell & Baumgartner 2019). The cost of clean–up would be significant.

- Eradication and control of SRDV, KIRV or LMBV, are expected to cause minor impacts at the national level.

The effect on domestic trade of industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Movement restriction orders, if put in place, would have indirect impacts on other industries such as seafood suppliers, commercial wild catch fisheries, and bait fisheries, due to the current host range of SRDV, KIRV and LMBV, including barramundi koi and common carp.
- Industries supplying inputs into the affected regions may suffer losses. For example, where farm production is haltered or decreased, feed companies would be impacted by reduced feed purchases.
- SRDV, KIRV or LMBV infected fish may show clinical signs which would affect their marketability.
- Should SRDV, KIRV or LMBV, establish and spread, there would likely be minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Whilst SRDV, KIRV or LMBV, are not WOAH listed, several other species and strains of ranaviruses are. Importing countries may have requirements for live, fresh or frozen species susceptible to ranaviruses.
- If SRDV, KIRV or LMBV, were to become established, Australia could use zoning to maintain access to international markets for live susceptible species.
- The impact of SRDV, KIRV or LMBV, establishment and spread on international trade is likely to be minor at the district or region level.

The effect on the environment, including biodiversity, endangered species, and the integrity of ecosystems

- The known host range for SRDV, LMBV and KIRV is narrow. Only LMBV has been reported in wild fish.
- There are no species listed as engendered in Australia that are related to species known to be susceptible to SRDV, KIRV or LMBV.
- The impacts of SRDV establishment and spread on environmental biodiversity are likely to be minor at the district or region level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Fish susceptible to SRDV, KIRV or LMBV are recreationally fished in Australia and could be affected by mortalities and movement restriction areas put in place, which may impact social amenity.

- In local areas where aquaculture is a major industry, a SRDV, KIRV or LMBV outbreak will likely impact communities by causing loss of business, or welfare concerns.
- If KIRV were to cause mortalities in the wild carp, large scale mortalities of carp may have a detrimental effect on social amenity if dead fish are not effectively removed and disposed of.
- The social impacts of SRDV, KIRV or LMBV, establishment and spread is expected to be minor at the district or regional level.

Table 20 shows the individual impact scores for each criteria (determined using Figure 4) for establishment and spread of SRDV, KIRV or LMBV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section Determining impacts for detailed methodology).

Table 20 Overall impact of establishment and spread of SRDV, KIRV and LMBV, for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Minor	E
	The environment (native animals/plants, and non-living environment)	National	Minor	E
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	District or region	Minor	C
	Environment (biodiversity, endangered species and the integrity of ecosystems)	District or region	Minor	C
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	C

Conclusion
The overall impact of establishment and spread of SRDV, KIRV or LMBV, was estimated to be **moderate**.

10.3.5 Determination of likely consequences of the outbreak scenario

The likely consequence of the outbreak scenario for SRDV, KIRV or LMBV, in each exposure group was determined by combining the partial likelihoods of establishment and spread, with the overall impact (using the matrix in Figure 6) and found to be:

- Australian ornamental fish – **Negligible**.
- Farmed foodfish – **Low**.
- Wild fish – **Low**.

10.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of SRDV, KIRV or LMBV, for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequence using matric in Figure 7 and found to be:

- Australian ornamental fish – **Negligible**.
- Farmed foodfish – **Negligible**.
- Wild fish – **Negligible**.

10.3.7 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 7.

The overall annual risk associated with SRDV, KIRV or LMBV, was found to be **negligible**.

Therefore, as the overall annual risk achieves Australia's ALOP, specific biosecurity measures are not considered necessary for this hazard.

11 Viral haemorrhagic septicaemia virus

11.1 Background

Viral haemorrhagic septicaemia virus (VHSV), also known as Novirhabdovirus piscine, is the aetiological agent of viral haemorrhagic septicaemia (VHS) (Ghittino 1965; WOAH 2023f). VHS is a serious disease that causes high mortalities of both farmed and wild fish in freshwater and marine environments (WOAH 2023f). It is named for the hallmark haemorrhaging that occurs in infected fish, especially in *Oncorhynchus mykiss* (rainbow trout). VHSV is classified in the genus Novirhabdovirus and family Rhabdoviridae (ICTV 2022).

The first records of a disease with clinical signs similar to VHS date back to 1938 in Germany, where a syndrome called Nierenschwellung (kidney swelling) was described in rainbow trout (Skall, Olesen & Mellergaard 2005b). In the 1950s, the syndrome appeared in Danish freshwater rainbow trout farms and a virus aetiology was confirmed (Jensen 1965). Infections with VHSV have since been detected in over 100 fish species in at least 30 countries in Europe, North America and North Asia (Batts et al. 2020; CEFAS 2022; WOAH 2023f).

Infection with VHSV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (WOAH 2023f) and is listed on Australia's *National list of reportable diseases of aquatic animals* (AHC 2021). Australia has a long history of passive surveillance and a strong system in place to detect incursions. VHSV is considered exotic to Australia.

11.2 Technical information

The technical information in this section will be used to make a conclusion about whether risk assessment of VHSV is warranted.

11.2.1 Agent properties

VHSV is an enveloped, bullet-shaped virion, approximately 70 nm in diameter and 180 nm in length, that encapsulates a negative-sense, single-stranded RNA segment (WOAH 2023f). VHSV is classified by the International Committee on Taxonomy of Viruses in the genus Novirhabdovirus and family Rhabdoviridae (ICTV 2022). Genotyping based on the nucleotide sequences of nucleoprotein and glycoprotein genes has further classified VHSV isolates into 4 major genotypes (I, II, III and IV) and 9 subtypes (Ia–Ie and IVa–IVd) that generally correlates with the geographical origin of the isolate (Einer-Jensen et al. 2004; Snow et al. 2004).

Genotype Ia contains almost all of the VHSV isolates causing outbreaks in European rainbow trout (Einer-Jensen et al. 2004; Snow et al. 2004). It also contains VHSV isolates detected from other fish species in Europe, both freshwater and marine, such as *Scophthalmus maximus* (turbot) (Schlotfeldt et al. 1991), *Salmo trutta* (brown trout) (de Kinkelin & Le Berr 1977) and *Esox lucius* (pike) (Jonstrup et al. 2009). The isolates in genotype Ib were collected from fish in the marine environment in the Baltic Sea, Kattegat, Skagerrak, the North Sea, the English Channel and Japan (Einer-Jensen et al. 2004; Nishizawa et al. 2002; Snow et al. 2004). Genotype Ic consists of isolates detected in Austria, Denmark and Germany (Jonstrup et al. 2009). The isolates in genotype Id are from outbreaks in

rainbow trout in Finland and other Scandinavian countries (Raja-Halli et al. 2006). Genotype Ie contains isolates from both freshwater and marine, farmed and wild fish in Georgia, Republic of Türkiye and the Black Sea (Altuntas & Ogut 2010; Einer-Jensen et al. 2004; Jonstrup et al. 2009; Nishizawa et al. 2006).

Genotype II has been predominantly isolated from wild marine fish such as *Clupea harengus* (Atlantic herring) from the Baltic Sea and *Lampetra fluviatilis* (lamprey) from the Kalajoki and Lestijoki rivers (Gadd et al. 2010; Gadd et al. 2011).

The isolates included in genotype III were collected from both farmed and wild fish in the North Atlantic Sea, the North Sea, the coastal waters of the United Kingdom (UK), Ireland and the British Isles (Dale et al. 2009; Lopez-Vazquez et al. 2006).

Genotype IVa has been detected in fish from coastal waters of North America and in South Korea and Japan (Garver et al. 2013; Meyers, Short & Lipson 1999; Meyers & Winton 1995; Ogut & Altuntas 2014). The isolates in genotype IVb were collected from freshwater fish from the North American Laurentian Great Lakes region (Gagné et al. 2007; Thompson et al. 2011; USGS 2008). The isolates included in genotype IVc were from fish in estuarine waters of New Brunswick and Nova Scotia, Canada (Faisal et al. 2012; Gagné et al. 2007; Stepien et al. 2015). Genotype IVd isolates were detected in Iceland in wild and farmed *Cyclopterus lumpus* (lumpfish) (Gudmundsdóttir et al. 2019).

Virus isolates show varied virulence depending on the fish species. For example, VHSV isolates originating from wild marine fish show no to low pathogenicity to rainbow trout and *Salmo salar* (Atlantic salmon) (Skall, Olesen & Mellergaard 2005b). In an immersion trial, a Norwegian isolate (genotype III) produced 70% mortality in rainbow trout whereas no mortality was observed in Atlantic salmon (Dale et al. 2009). Experimental infection of rainbow trout using a VHSV genotype IVa isolate demonstrated no to low pathogenicity whereas genotype I and III isolates caused high levels of mortality (Dixon et al. 1997; Follett et al. 1997; Skall et al. 2004; Winton et al. 1991).

VHSV survival outside of the host depends on the isolate, temperature and other environmental conditions (WOAH 2023f). The optimum water temperature for VHSV is typically 1–12°C with virulence greatly reduced at or above 20°C ((Jorgensen 1973a) cited in (Meyers & Winton 1995))(Jorgensen 1982b; Smail 1999). Genotype IVb has been isolated between 12–18°C (Kane-Sutton et al. 2010). Outbreaks of VHSV occur during all seasons but are most common in the Northern Hemisphere during spring when water temperatures are rising or fluctuating (WOAH 2023f).

VHSV can remain infectious in fresh and sea water for extended periods. For example, one study showed the virus survived in fresh water for 28–35 days at 4°C ((Parry & Dixon 1997) cited in (WOAH 2023f)). Hawley & Garver (2008) reported that in raw sea water, the 99.9% inactivation time of 4 VHSV isolates ranged from 13 days at 4°C to 1.5 days at 20°C (Hawley & Garver 2008). In filtered sea water, the 99.9% inactivation times ranged from 8.7 days at 4°C to 0.5 days at 20°C. When the viral isolates were incubated in in raw fresh water, 99.9% inactivation ranged from 40 days at 4°C to less than 1 day at 30°C. In filtered fresh water, the 99.9% inactivation times ranged from >489 days at 4°C to <2 days at 30°C. (Hawley & Garver 2008). In another study using filtered sea water at 15°C, the infectivity of VHSV was reduced by 50% after 10 hours but could still be recovered after 40 hours (Kocan, Hershberger & Elder 2001). The virus remained stable for a longer time if sterile organic

materials were added to the water, such as ovarian fluids or bovine serum (Kocan, Hershberger & Elder 2001). VHSV was shown to survive in filtered river water for >65 days at 4°C and 10°C and 49–56 days in water at 25°C (Joiner et al. 2021). Survival in unfiltered river water ranged from >84 days at 4°C to 28 days at 25°C (Joiner et al. 2021).

VHSV survives freezing at –20°C (Wolf 1988). However, VHSV-infected fish subjected to the commercial freezing process (core block temperature of –24°C) had a 90% reduction in viral titre after the tissue was thawed (Arkush et al. 2006).

VHSV is sensitive to a wide range of disinfectants including 2% formalin, sodium hydroxide, chlorine, sodium hypochlorite, iodine and Virkon S ((Ahne 1982) cited in (Olesen 1998))(Smail 1999; Wolf 1988). The virus is also sensitive to ultraviolet light, bacterial degradation in sediments and enzymatic activity in decomposing fish (Oye & Rimstad 2001; WOAH 2023f).

11.2.2 Epidemiology

Host range

VHSV has a wide host range (Batts et al. 2020; WOAH 2023f) as summarised in Table 21 and presented in full in Appendix C.

Table 21 Genera susceptible to VHSV

Environment	Family	Genus	Permitted import	VHSV	
				Susceptible to infection	Positive PCR; no active infection
Marine	Alosidae	*Sardinops*	No	N	-
	Argentinidae	*Argentia*	No	N	-
	Anoplopomatidae	*Anoplopoma*	No	-	N
	Carangidae	*Seriola*	No	N	-
	Cyclopteridae	*Cyclopterus*	No	N	-
	Epinephelidae	*Epinephelus*	No	E	-
	Gadidae	*Melanogrammus*	No	N	-
		Micromesistius	No	N	-
		Trisopterus	No	N	-
	Gaidropsaridae	*Enchelyopus*	No	N	-
	Labridae	*Centrolabrus*	Yes	N, E	-
		Ctenolabrus	Yes	N	-
		Labrus	Yes	N	-
		Symphodus	Yes	N	-
	Liparidae	*Liparis*	No	N	-
	Mullidae	*Mullus*	Yes	N	-
	Ophidiidae	*Hoplobrotula*	No	N	-
	Paralichthyidae	*Paralichthys*	No	N	-

Environment	Family	Genus	Permitted import	VHSV	
				Susceptible to infection	Positive PCR; no active infection
	Pleuronectidae	*Limanda*	No	N	-
		Reinhardtius	No	N	-
	Rajidae	*Raja*	No	N	-
	Sciaenidae	*Larimichthys*	No	N	-
	Scombridae	*Scomber*	No	N	-
	Scorpaenidae	*Scorpaena*	No	N	-
	Scyliorhinidae	*Scyliorhinus*	No	N	-
	Soleidae	*Solea*	No	N	-
	Stromateidae	*Pampus*	No	N	-
	Triglidae	*Eutrigla*	No	N	-
	Uranoscopidae	*Uranoscopus*	No	N	-
Euryhaline	Alosidae	*Alosa*	n/a	N	-
		Sardina	n/a	N	-
	Ammodytidae	*Ammodytes*	n/a	N	-
	Anguillidae	*Anguilla*	n/a	N	-
	Belonidae	*Belone*	n/a	N	-
	Carangidae	*Trachurus*	n/a	N	-
	Clupeidae	*Clupea*	n/a	N	-
		Sprattus	n/a	N	-
	Dorosomatidae	*Dorosoma*	n/a	N	-
	Embiotocidae	*Cymatogaster*	n/a	N, E	-
	Engraulidae	*Engraulis*	n/a	N	-
	Fundulidae	*Fundulus*	n/a	N	-
	Gadidae	*Gadiculus*	n/a	N	-
		Gadus	n/a	N	-
		Merlangius	n/a	N	-
	Gasterosteidae	*Gasterosteus*	n/a	N	-
	Gobiidae	*Neogobius*	n/a	N	-
		Pomatoschistus	n/a	N	-
		Rhinogobius	n/a	E	-
	Merlucciidae	*Merluccius*	n/a	N	-
	Moronidae	*Dicentrarchus*	n/a	E	-
		Morone	n/a	N	-
	Mugilidae	*Mugil*	n/a	N	-
	Osmeridae	*Hypomesus*	n/a	N	-
		Thaleichthys	n/a	N	-

Environment	Family	Genus	Permitted import	VHSV	
				Susceptible to infection	Positive PCR; no active infection
	Petromyzontidae	*Lampetra*	n/a	N	-
	Pleuronectidae	*Glyptocephalus*	n/a	N	-
		Hippoglossus	n/a	N	-
		Platichthys	n/a	N	-
		Pleuronectes	n/a	N	-
	Salmonidae	*Oncorhynchus*	n/a	N, E	-
		Salmo	n/a	N	-
		Salvelinus	n/a	N	-
	Scophthalmidae	*Scophthalmus*	n/a	N, E	-
	Sparidae	*Acanthopagrus*	n/a	E	-
		Sparus	n/a	-	N
	Trichiuridae	*Trichiurus*	n/a	N	-
Freshwater	Acipenseridae	*Acipenser*	n/a	-	E
	Adrianichthyidae	*Oryzias*	n/a	E	-
	Anguillidae	*Anguilla*	n/a	N	-
		Ictalurus	n/a	N	-
	Catostomidae	*Catostomus*	n/a	N	-
		Moxostoma	n/a	N	-
	Centrarchidae	*Ambloplites*	n/a	N	-
		Lepomis	n/a	N	-
		Micropterus	n/a	N	-
		Pomoxis	n/a	N	-
	Cottidae	*Cottus*	n/a	E	-
	Danionidae	*Danio*	n/a	E	-
	Esocidae	*Esox*	n/a	N	-
	Fundulidae	*Fundulus*	n/a	N	-
	Ictaluridae	*Ameiurus*	n/a	N	-
	Leuciscidae	*Notemigonus*	n/a	E	-
		Notropis	n/a	N	-
		Pimephales	n/a	N, E	-
		Pseudochondrostoma	n/a	-	N
		Semotilus	n/a	N	-
	Lotidae	*Lota*	n/a	N	-
	Percidae	*Perca*	n/a	N	-
		Sander	n/a	N	-
	Percopsidae	*Percopsis*	n/a	N	-

Environment	Family	Genus	Permitted import	VHSV	
				Susceptible to infection	Positive PCR; no active infection
	Salmonidae	*Coregonus*	n/a	N, E	-
		Thymallus	n/a	N	-
	Sciaenidae	*Aplodinotus*	n/a	N	-

Permitted import Included on the department's List of permitted live marine ornamental fish suitable for import. **n/a** Not applicable, these species are not included on the department's List of permitted live marine ornamental fish suitable for import and are outside the scope of this review. **N** Susceptible to infection by natural exposure. **E** Susceptible to infection by experimental exposure.

VHSV may infect all life stages of fish (Munro & Gregory 2010; WOAH 2023f). Host susceptibility is influenced by multiple factors including virus isolate, temperature, fish stock, species, age, size and immune status (Hershberger et al. 2007; Hershberger et al. 1999; Lorenzen & Lapatra 1999).

Detection of VHSV in marine ornamental species currently permitted import into Australia is limited to *Labridae* (wrasse) species *Centrolabrus exoletus* (rock cook wrasse), *Ctenolabrus rupestris* (goldsinny wrasse), *Labrus bergylta* (ballan wrasse), *Labrus mixtus* (cuckoo wrasse), *Mullus barbatus* (red mullet) and *Symphodus melops* (corkwing wrasse) (Munro et al. 2015; Ogut & Altuntas 2014). Although, all mentioned wrasse species are commonly used in *Salmonidae* (salmonid) aquaculture as cleaner fish and are not likely to be imported as ornamental fish (Skiftesvik et al. ; Treasurer 2002). *Macropharyngodon geoffroy* (leopard wrasse) may also be susceptible as cell lines derived from its skin and fins were found to be permissible to VHSV infection (Ma et al. 2013).

Geographical distribution

VHSV is considered endemic throughout the Northern Hemisphere (Batts et al. 2020). European countries where VHSV has been reported include Austria, Belgium, Denmark, Finland, France, Germany, Iceland, Italy, the Netherlands, Norway, Poland, Sweden and Switzerland (Gudmundsdóttir et al. 2019; Jensen 1965; Olesen 1998; Raja-Halli et al. 2006). The virus has also been isolated from Canada (Meyers & Winton 1995), Georgia (Batts et al. 2020), Ireland (Schlotfeldt et al. 1991), Japan (Isshiki et al. 2001), Republic of Korea (Kim et al. 2003), Republic of Türkiye (Batts et al. 2020), UK (Schlotfeldt et al. 1991; Smail 2000) and United States of America (USA) (Hopper 1989).

Prevalence

Marine ornamental fish

Natural infection was reported in wild ballan wrasse, corkwing wrasse, cuckoo wrasse, goldsinny wrasse, and rock cook wrasse, all captured and held at a marine hatchery in the Shetland Isles, Scotland but prevalence was not estimated (Munro et al. 2015).

Other fish

Surveys of the North Sea, Skagerrak, Kattegat, the Bay of Århus and the Baltic Sea during 1998–2002 detected VHSV at a prevalence of 6.7% (n=5699) in Atlantic herring, 11% (n=3038) in *Sprattus sprattus* (sprat), 3.4% (n=1,272) in *Limanda limanda* (common dab), 8.4% (n=431) in *Platichthys flesus* (European flounder), 2.8% (n=531) in *Pleuronectes platessus* (European plaice), 14.3% (n=210) in *Pomatoschistus minutus* (sand goby), 2.4% (n=252) in *Ammodytes* species (sand lances) and 0.2% (n=2039) in *Gadus morhua* (Atlantic cod) (Skall, Olesen & Mellergaard 2005a). A

2009–11 survey in the Black Sea for VHSV in marine fish found a prevalence of 30% in *Uranoscopus scaber* (Atlantic stargazer) (n=33 pools), 6.5% in *Trachurus mediterraneus* (Mediterranean horse mackerel) (n=168 pools), 2.3% in *Scorpaena porcus* (black scorpionfish) (n=43 pools) and 1% in *Mullus barbatus* (red mullet) (n=100 pools) (Ogut & Altuntas 2014). A survey of VHSV was conducted on wild fish species collected in 8 coastal areas of Japan in 2001 and virus was isolated from 10% (n=160) of *Paralichthys olivaceus* (Bastard halibut) and 2% (n=52) of *Ammodytes personatus* (sand eel) (Watanabe et al. 2002). In Finland, VHSV was detected in 6.7% of *Clupea harengus membras* (Baltic herring) (n=758 pools where each pool contained 10 Baltic herring) collected during 2004–06 (Gadd et al. 2011). In 2013, an epidemiological investigation of 1400 marine fish (covering 6 species) collected in Shetland, Scotland detected VHSV at 8% prevalence (n=140 pools where each pool was 10 fish) (Wallace et al. 2015).

In 2020, 11 new outbreaks of VHSV were reported in Europe with 5 in Germany, 5 in Belgium, 2 in France, 1 in Czech Republic and 1 in Italy (EURL for Fish and Crustacean Diseases 2021). Additionally, in 2020 there were 13 VHSV-infected fish farms (subject to minimum control measures) in Italy (n=816), 1 farm in Austria (n=4862), 2 farms in Belgium (n=100), 9 farms in Germany (n=10,813), 3 farms in Slovenia (n=303) as well as 2 VHSV-infected fish farms subject to an eradication programme in Belgium (n=100) (EURL for Fish and Crustacean Diseases 2021).

Surveillance conducted to monitor VHSV in the State of Michigan, USA during 2005–10 on 96,228 fish (73 species) detected virus at 1.6% prevalence in 19 fish species (n=1823 cases where a case is defined as a group of fish (wild or farmed) of the same species, source, and date of submission) (Faisal et al. 2012). In 2006, a survey of 1,011 apparently healthy fish representing 20 species from 19 different bodies of water throughout New York State, USA identified VHSV positives in 8 species (Frattini et al. 2011). Analysis of 1,428 fish (32 species) caught from the Great Lakes region, USA between 2006 and 2007 detected VHSV in 28% of the fish (n=1428) (Hope et al. 2010).

Mortalities

Mortality rates vary by fish species, age, environmental conditions and the virus isolate (LaPatra et al. 2016). Fish deaths from VHSV have been reported to occur in water temperatures between 1–15°C (Wolf 1988) and to peak at 9–12°C (Smail 1999). At water temperatures between 15–18°C, the disease is generally acute with low levels of mortality. At low water temperatures (1–5°C) the disease is generally extended with low daily mortality but high accumulated mortality (WOAH 2023f).

Marine ornamental fish

In 2012, a wild wrasse population (approximately 10 000 wrasse) being held at a marine hatchery in the Shetland Isles, Scotland, prior to being deployed for the biological control of parasites on marine pen Atlantic salmon, suffered a VHSV outbreak (genotype III, the European marine strain) with a daily mortality rate of 1.4%. However, due to mixed infection in the caught wrasse, the mortalities cannot be definitively associated with VHSV (Munro et al. 2015).

Other fish

Mortality often reaches 80–100% in rainbow trout fry and fingerlings and less in older fish, typically 25–75% depending on stressors present (Meyers & Winton 1995; Skall, Olesen & Mellergaard 2005b; Wolf 1988). Fish deaths due to a VHSV outbreak in farmed rainbow trout in the UK in 2006 peaked at approximately 2000 per day (Stone et al. 2008). In January–June 2021, VHSV outbreaks in Iran in rainbow trout resulted in 565,867 fish being slaughtered and an additional 101,383 deaths (WOAH

2022d). During the same period, a VHSV infection in brown trout in the Czech Republic led to 14,500 mortalities (WOAH 2022d).

Mass mortality of *Sardinops sagax* (sardine) has frequently been reported along coastal British Colombia since 1998 due to VHSV epizootics with die-offs estimated to be in excess of 5,000 metric tonnes and spreading over distances of 15 km (Garver et al. 2013). In the Bay of Quinte, Canada, VHSV infection of *Aplodinotus grunniens* (freshwater drum) in 2005 caused mass mortality, resulting in an estimated 100 metric tonnes of dead fish (Lumsden et al. 2007). In 2006, a large mortality of several thousand *Neogobius melanostomus* (round goby) occurred in New York waters of the St. Lawrence River and Lake Ontario due to VHSV (Groocock et al. 2007). VHSV caused several outbreaks in the Great Lakes, USA watershed between 2005–08 resulting in large-scale fish kills (no numbers given) (Thompson et al. 2011).

Transmission

Transmission occurs horizontally either by direct contact with diseased fish or indirect contact via contaminated water and equipment (Matejusova et al. 2016; Meyers & Winton 1995; Smail 1999). Oral transmission has also been demonstrated by feeding infected fish and tissue homogenates to healthy fish (Getchell et al. 2013; Meyers & Winton 1995; Oidtmann et al. 2011a). Vertical transmission within the egg has yet to be demonstrated (Munro & Gregory 2010) but VHSV has been isolated from ovarian fluid, ovaries and milt ((Jorgensen 1973b) cited in (Munro & Gregory 2010))(Al-Hussinee et al. 2010; Eaton et al. 1991; Tuttle-Lau, Phillips & Gaikowski 2009).

Virus can be shed from infected fish via the urine, faeces and reproductive fluids ((Jorgensen 1970) cited in (Meyers & Winton 1995))(Neukirch & Glass 1984). The shedding rate of VHSV from experimentally infected *Clupea pallasii* (Pacific herring) (by immersion) was estimated at a maximum of $1.8–5.0 \times 10^8$ PFU/fish/day (Hershberger et al. 2010a). The shed virus was first detected in the flow-through tanks 4–5 days post infection (dpi), which preceded initial mortality by 2 days, peaked after 6–10 days and was no longer detected after 16 days (Hershberger et al. 2010a). Experimentally infected *Esox masquinongy* (muskellunge) (by immersion) shed upwards of 1.36×10^5 PFU/fish/hour from 3 weeks post-exposure up to 15 weeks (Kim & Faisal 2012).

Fish surviving the disease may become carriers of the virus and a source of infection (Kocan et al. 2001; Neukirch & Glass 1984; Skall, Olesen & Mellergaard 2005b). For example, rainbow trout that had survived an experimental VHSV infection shed infectious virus via urine for more than 30 days after a secondary challenge infection without any clinical signs of infection (Neukirch & Glass 1984). Genotype IVa was shown to persist for at least 224 days in healthy Pacific herring following infection (Hershberger et al. 2010b). In clinically healthy rainbow trout, VHSV persisted for up to 14 weeks at 5°C (Jorgensen 1982b).

Susceptible hosts can develop adaptive immunity after surviving a VHSV infection. This is supported by studies that showed a greater resistance to VHSV among older age fish (Hershberger et al. 2001; Hershberger et al. 1999) and a resistance among fish that survived previous VHSV outbreaks or have been vaccinated (Hershberger et al. 2007; Kocan et al. 2001; Lorenzen & Lapatra 1999). Stressors that have been correlated with VHSV infections in survivor or naive fish include poor water quality, high fish density, high feeding rate, rough handling of fish, capture of fish, spawning and infection with other disease agents (Meyers et al. 1994; Meyers & Winton 1995; WOAH 2023f). For example, several VHSV infections among Pacific herring and sardines in the Northeast Pacific Ocean have been

associated with stocks of fish that encountered abnormally low water temperatures, which presumably reduced the ability of the fish immune system to resist infection (Hedrick et al. 2003).

Birds may act as mechanical vectors of VHSV as the virus could be re-isolated from regurgitated infected fish from *Ardea cinerea* (heron) (Peters & Neukirch 1986). VHSV has also been isolated from a range of other animals that may act as vectors such as *Myzobdella lugubris* (piscicolid leech) (Faisal & Schulz 2009), *Diporeia* species (amphipods) (Faisal & Winters 2011), *Moina macrocopa* (water flea) (Ito & Olesen 2017), *Chelra serpentina* (common snapping turtle), *Trachemys scripta elegans* (red-eared slider) and *Grapetemys geographicas* (northern map turtle) (Goodwin & Merry 2011). Transmission from fomites and sediment may occur as the virus remained infectious for several weeks after being dried on stainless steel and in various soil types (Joiner et al. 2021). Laboratory studies have also demonstrated that VHSV can adhere and remain infectious on plastic, aluminium and fishing line for at least 10 days when wet but only 1 day when dry (Pham et al. 2012).

Mechanism of spread

VHSV is considered to have originated in marine fish species and then spread to the freshwater environment (Einer-Jensen et al. 2004; Einer-Jensen, Winton & Lorenzen 2005; Snow et al. 2004; Stone, Way & Dixon 1997). This spread may have happened by the feeding of raw infected marine fish to cultured freshwater fish (Dixon 1999; Meyers & Winton 1995; Stone, Way & Dixon 1997). The movement of live fish has also contributed to the spread of virus from marine to fresh water and vice versa (Castric & de Kinkelin 1980). Aquatic experts estimated the movement of live fish and eggs was the highest risk factor for spreading VHSV with exposure to infected water also an important pathway (Oidtmann et al. 2014). For example, an initial outbreak of VHSV at a seawater site in Norway rearing rainbow trout was detected in 3 neighbouring sea sites within 3–4 months (Dale et al. 2009). At salmonid net pen sites, herring and sardines are often observed swimming around and within the enclosures and if infected with VHSV can shed the virus to infect the farmed salmonids (Garver et al. 2013). Transport water may also contribute to spread of VHSV as it has been shown that water containing healthy but infected wild Pacific herring that were being transferred to a laboratory contained VHSV at concentrations of 1.6×10^2–1.7×10^3 PFU/ml (Kocan et al. 2001).

Based on experience with VHSV in Europe and North America, an international panel of fish health experts identified risk factors that have played a role in the emergence and spread of VHSV in the Great Lakes basin, USA, that are likely applicable to other geographical locations (VHSV Expert Panel and Working Group 2010). These factors included the presence of VHSV-susceptible species, water temperature, proximity to known VHSV-positive areas, untested shipments of live or frozen fish from infected zones, insufficient regulatory infrastructure for oversight of fish health, and uncontrolled exposure to fomites associated with boats and equipment or fish wastes from known VHSV-positive areas (VHSV Expert Panel and Working Group 2010).

Infectious dose

Goldsinny wrasse (mean weight 10 g) challenged by immersion with 1×10^5 $TCID_{50}$/mL VHSV (genotype III) for 1 hour resulted in infection (Matejusova et al. 2016). *Coregonus* fish (3 cm length) infected by immersion for 1 hour in 10^4 $TCID_{50}$/mL at 11–12°C resulted in morbidity and mortality (Ahne & Thomsen 1985). Bath exposure of turbot (mean weight 8 g) for 3 hours to 8×10^4 $TCID_{50}$/mL VHSV caused 63–74% mortality (Snow & Smail 1999). Outbreaks of acute disease, accompanied by mortality and viral shedding, were initiated after bath exposure of Pacific herring (mean length 100 mm; 1+ year old) to concentrations of VHSV at 10^1 PFU/mL for 24 hours or 10^2–10^3 PFU/mL for

1 hour (Hershberger et al. 2010a; Kocan et al. 1997). Pacific herring (mean weight 14 g) intraperitoneally injected with 19, 0.7 and 0.07 PFU/fish caused infection rates of 100%, 75% and 38%, respectively (Hershberger et al. 2011). The median lethal dose of infection (LD50) of genotype IVb for juvenile muskellunge by intraperitoneal injection (weight 17.5 g) was 2.2 PFU and for immersion challenge (weight 0.7 g) was 1.7×10^4 PFU/mL (Kim & Faisal 2010).

Bath challenge experiments using a UK VHSV isolate at 10 $TCID_{50}$/mL for 4 hours could induce mortality in rainbow trout (weight 4.6–15.8 g) whereas a concentration of 10^3 $TCID_{50}$/mL was required to cause mortality in brown trout (mean weight 5 g) (Dixon, Joiner & Way 2007). Evensen et al (1994) bath challenged rainbow trout (15 cm length) with three concentrations of VHSV for 1 hour and observed 44% mortality at 14 dpi in the group at the lowest dose of 10^2 $TCID_{50}$/mL (Evensen et al. 1994). Bath exposure of rainbow trout (weight 20–30 g) with 3.5×10^3 $TCID_{50}$/mL for 2 hours led to morbidity and mortality (Jonstrup et al. 2013). Juvenile rainbow trout (≤2 g) immersed for 1 hour in 10^5 PFU/mL showed mortality but none was seen with a dose of 10^3 PFU/mL (Meyers & Winton 1995). Rainbow trout fry (mean weight 4.4 g) fed infected viscera (1.41×10^4 $TCID_{50}$/mL) or brain and gill (4.45×10^3 $TCID_{50}$/mL) homogenates resulted in 100% mortality after 7–11 dpi (Oidtmann et al. 2011a).

11.2.3 Pathogenesis

Tissue tropism

The primary portal of entry is considered to be the epithelial tissues of the gills or skin, especially at the base of the fins, with spread to other tissues via blood (Harmache et al. 2006; Neukirch 1984). Target organs are anterior kidney, heart and spleen, as these are the sites in which virus is most abundant (Smail 1999; Wolf 1988). VHSV has also been detected in ovarian fluid (Hopper 1989; Meyers & Winton 1995), ovaries and testis (Al-Hussinee et al. 2010; Al-Hussinee et al. 2011), skin (Meyers et al. 1994; Yamamoto, Batts & Winton 1992), fins (Cornwell & Bellmund 2013), gills (Al-Hussinee et al. 2010; Oidtmann et al. 2011b), liver (Evensen et al. 1994) and digestive tract (Al-Hussinee et al. 2010; Schonherz et al. 2012). In chronic stages, virus titres can become high in the brain (Castric & de Kinkelin 1980; Duesund et al. 2010; Hershberger et al. 2010b; Neukirch 1984; Oidtmann et al. 2011b).

Tissue titre

Viscera from infected wild Pacific herring collected in Washington, USA had VHSV titres ranging from 2×10^3–9×10^4 PFU/mL (Meyers & Winton 1995). Wild Pacific herring from Hoonah Harbour, Alaska had titres of VHSV ranging from 3.16×10^4–5.62×10^7 $TCID_{50}$/mL (Meyers & Winton 1995). Healthy but infected wild Pacific herring and *Ammodytes hexapterus* (Pacific sand lance) that were captured and confined to a laboratory developed VHSV infections with virus titres reaching 10^8 PFU/g and 10^6 PFU/g of pooled spleen and kidney tissue, respectively (Kocan et al. 2001). Experimentally infected (by immersion) Pacific herring (age 1+ year) resulted in VHSV titres of 1.2×10^3–3.3×10^7 PFU/g of pooled kidney and spleen and 7.1×10^3–2.2×10^7 PFU/g of brain in dead fish (Hershberger et al. 2010b). The VHSV titre in pooled kidney and spleen tissue from dead Pacific herring experimentally infected (by immersion) was 5.7×10^5 PFU/g and 1.2×10^4 PFU/g in survivors (Hershberger et al. 2010a). VHSV titres from infected sardines ranged from 1.4×10^2–1.6×10^6 PFU/g of tissue (Traxler, Kieser & Richard 1999). Pooled kidneys, liver and spleens from experimentally infected *Coregonus* fish had a virus titre of $10^{6.8}$ $TCID_{50}$/g (Ahne & Thomsen 1985).

Seawater rainbow trout that were either injected with or immersed in VHSV recorded virus titres of 8×10^6–1×10^8 PFU/g and 6×10^5–3×10^8 PFU/g pooled kidney and spleen tissue, respectively (Castric & de Kinkelin 1980). The results were similar for infected freshwater rainbow trout, ranging between 4×10^6–2×10^7 PFU/g of tissue for injected fish and between 2×10^7–2×10^8 PFU/g for bath exposed fish (Castric & de Kinkelin 1980). VHSV titres from rainbow trout infected by immersion challenge were 2.11×10^7 $TCID_{50}$/g internal organs (pooled kidney, spleen and liver), 6.67×10^6 $TCID_{50}$/g brain and gill and 2.81×10^6 $TCID_{50}$/g muscle tissue (Oidtmann et al. 2011a). In muscle and gill/brain tissue from infected market-size rainbow trout the VHSV titres were $10^{1.0}$–$10^{6.8}$ $TCID_{50}$/g before onset of clinical signs, $10^{0.5}$–$10^{5.8}$ $TCID_{50}$/g in clinically affected fish, $10^{2.3}$–$10^{6.6}$ $TCID_{50}$/g in dead fish and $10^{0.5}$–$10^{6.7}$ $TCID_{50}$/g in clinically healthy fish still alive 6 weeks after challenge with VHSV (Oidtmann et al. 2011b). Titres in bath infected rainbow trout ranged from 2.7×10^2 to $>1 \times 10^6$ $TCID_{50}$/mL (Jonstrup et al. 2013). VHVS replication in excised skin of rainbow trout reached 10^9 PFU/g for genotype Ia and 10^4 PFU/g for genotype IVa (Yamamoto, Batts & Winton 1992). The viral titre in experimentally infected (by immersion) dead Atlantic salmon ranged from 4×10^5–1×10^7 PFU/g kidney tissue (Lovy et al. 2013). In surviving fish 10 weeks later, the titres were higher than 1×10^6 PFU/g kidney tissue (Lovy et al. 2013).

11.2.4 Diagnosis

Clinical signs

Clinical signs characteristic of infection with VHSV include rapid onset of mortality, lethargy, darkening of the skin, exophthalmia, anaemia (pale gills), abnormal swimming such as flashing and spiralling, a distended abdomen due to oedema in the peritoneal cavity and haemorrhages at the base of the fins or in the gills, eyes or skin (WOAH 2023f). In aquaculture, fish may accumulate near the outlet of the pond or at the sides or bottom of the tank (LaPatra et al. 2016). In some cases, no clinical signs accompany infection (Enzmann & Konrad 1985; Hedrick et al. 2003; Jorgensen 1982a; King et al. 2001; Meyers, Short & Lipson 1999; Meyers & Winton 1995; Mortensen et al. 1999).

Pathology

Pale viscera and generalised congestion and petechial haemorrhaging in the skin, muscle tissue (especially in dorsal muscles), internal organs and meninges are observed in infected fish (Meyers & Winton 1995; Skall, Olesen & Mellergaard 2005b; WOAH 2023f). The kidney, liver, spleen and haematopoietic tissue typically show extensive multifocal necrosis and degeneration (WOAH 2023f). Necrosis has also been detected in the gills and heart (Isshiki et al. 2001; Kim & Faisal 2010). The spleen, liver and kidneys may be swollen (Batts et al. 2020; Isshiki et al. 2001; WOAH 2023f). Vasculitis (Evensen et al. 1994) and endocarditis (Dale et al. 2009) have been noted in some cases. In the central nervous system, multifocal necrosis in the brain, medulla and spinal cord and degeneration of peripheral nerves and optic nerves may occur (Batts et al. 2020; Dale et al. 2009; Evensen et al. 1994; Lumsden et al. 2007).

Testing

Chapter 2.3.10 of the WOAH Manual of diagnostic tests for aquatic animals (WOAH 2023f) provides details of the methods currently available for surveillance and confirmatory diagnosis of VHSV. Cell culture (Lorenzen, Carstensen & Olesen 1999; USGS 2007), real-time RT-PCR (Garver et al. 2011; Jonstrup et al. 2013) and conventional RT-PCR (Kim, Cuenca & Olesen 2018) with amplicon sequencing are the recommended methods for confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis for all life stages (WOAH 2022a).

Other testing methods to detect VHSV include immunohistochemistry (Evensen et al. 1994), indirect fluorescent assay (Lorenzen, Olesen & Jorgensen 1988), antigen enzyme-linked immunosorbent assay (Way & Dixon 1988) and histopathology (WOAH 2023f).

11.2.5 Treatment

There are currently no treatments for VHSV (WOAH 2023f).

11.2.6 Control and prevention

An eradication program to eliminate VHSV from Denmark was initiated in 1965 and resulted in the dramatic reduction in the number of infected rainbow trout farms from 400 to 26 by 1997 (Olesen 1998). Eradication involved draining, removal of all fish, cleaning and disinfection followed by repopulation with VHSV-free fish and water (Commission 2015; Olesen 1998). Fencing against birds and animals, separation between areas and effective prevention of the escape of farmed fish and the entrance of wild fish into farms were also applied (Olesen 1998). It has been noted that it can be difficult to eradicate VHSV in areas where it is endemic due to the lack of effective control of all aquaculture facilities in an area combined with a lack of financial assistance and organisation (Olesen 1998).

Similar biosecurity measures were implemented in USA hatcheries following VHSV outbreaks (Faisal et al. 2012). These included restrictions on broodstock collection locations, enhanced screening of broodstock as VHSV-negative prior to stocking, disinfection of eggs with iodophor, only accepting certified VHSV-free transfer fish and stringent equipment disinfection. If there was no information on the effectiveness of egg disinfection for the fish species then VHSV testing was required on pre-spawn adults, adults used in spawning, fry, and spring fingerlings. There may also be a strong tendency for fish to eliminate virus infections at warmer temperatures of 15–20°C provided there is no source for re-infection (Meyers & Winton 1995). Biosecurity measures implemented for imported baitfish required the fish be certified VHSV-negative and to be used within 14 days (Faisal et al. 2012). Tight controls on other fish imports can also prevent VHSV entering a country (Meyers & Winton 1995).

Upon confirmation of VHSV infection in wrasse species in a hatchery in Scotland, movement restrictions were immediately applied to live aquaculture animals, aquaculture animal products, aquaculture animal feed and equipment and a containment area was established around the infected site with the purpose of preventing the spread of the infection (Hall et al. 2013; Munro et al. 2015).

The disinfection of eggs with iodophor is common practice to prevent VHSV infections although it is not always reliable ((Jorgensen 1973b) cited in (Munro & Gregory 2010))(Bovo et al. 2005; WOAH 2023f). It was estimated that 1 out of 100 disinfected rainbow trout egg consignments would still lead to VHSV infections at receiving sites (Oidtmann et al. 2014).

A commercial vaccine for VHSV is not yet available (WOAH 2023f). Candidate vaccines have included killed vaccines, attenuated live vaccines, a recombinant vaccine in prokaryotic and eukaryotic expression systems, and DNA-based vaccines (Jorgensen 1982b; Lecocq-Xhonneus et al. 1994; Lorenzen et al. 2000; Souto et al. 2023; Sytandish et al. 2016). Studies into the selection of fish with increased resistance to VHSV began in the mid-1980s with rainbow trout (Chevassus & Dorson 1990;

Dorson et al. 1995; Henryon et al. 2002; Verrier et al. 2013); however, no resistant rainbow trout strains are commercially available (WOAH 2023f).

11.2.7 Impact of the disease

VHSV has the potential to cause significant losses in a broad range of hosts and an ability to spread rapidly (LaPatra et al. 2016). Consequently, VHSV outbreaks have had major economic impacts on recreational angling and aquaculture businesses rearing susceptible species (LaPatra et al. 2016). VHSV is one of the most economically important viral diseases in European salmonid farming causing estimated losses of £40 million per year in 1991 (Einer-Jensen et al. 2004)((Hill 1992) cited in (Skall, Olesen & Mellergaard 2005b)). In 2000, the economic loss of VHSV outbreaks on 2 Danish fish farms producing approximately 165 tonnes rainbow trout was estimated to be above €211,000 with an associated total mortality of 50% ((Nylin & Olesen 2001) cited in (Skall, Olesen & Mellergaard 2005b)). VHSV outbreaks occurring in wild populations may also contribute to population declines. For example, the isolation of VHSV from Pacific herring in Prince William Sound, Alaska in 1993 coincided with the disappearance of 83% of the predicted biomass of 134,133 metric tons of herring and a failed population recovery in subsequent years (Kocan et al. 1997; Marty et al. 2010).

11.2.8 Current biosecurity measures

There are no current biosecurity measures for live marine ornamental fish.

11.2.9 Conclusion

VHSV is present in exporting countries, is not present in Australia and is capable of causing adverse effects. In Australia, infection with VHSV is a nationally notifiable disease. Based on the preceding information, risk assessment is warranted.

11.3 Risk assessment

Based on chapter 2 and the technical information about VHSV presented in this chapter, the risk assessment was completed.

A summary of the risk assessment values are shown in Appendix D.

11.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for VHSV were that:

- This risk review is generic and therefore the entry assessment assumes that VHSV is present in all source countries.
- There are limited reports of VHSV infecting marine ornamental fish on the List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*) (refer Appendix A). Natural infection (genotype III) with mortalities was reported in wild *Labrus bergylta* (ballan wrasse), *Symphodus melops* (corkwing wrasse), *Labrus mixtus* (cuckoo wrasse), *Ctenolabrus rupestris* (goldsinny wrasse), and *Centrolabrus exoletus* (rock cook wrasse) captured and held at a marine hatchery in the Shetland Isles, Scotland (Munro et al. 2015). Experimentally, wrasse cell lines have also been shown to be permissible to VHSV infection (Ma et al. 2013).
- Wild *Mullus barbatus* (red mullet) collected from the southeastern Black Sea tested PCR-positive for VHSV (genotype Ie) (Ogut & Altuntas 2014).

- Prevalence of VHSV in marine ornamental fish is unknown. Prevalence in farmed salmonids can reach 100% and in wild fish populations can be up to 30%.
- VHSV can survive in seawater and freshwater for extended periods, particularly at low temperatures.
- The optimum water temperature for VHSV is typically 1–12°C with virulence greatly reduced at or above 20°C ((Jorgensen 1973a) cited in (Meyers & Winton 1995))(Jorgensen 1982b; Smail 1999).
- The viral load of VHSV in infected imported fish would likely be sufficient to cause infection in susceptible species.
- Pre-export inspection may detect ornamental fish showing clinical signs of infection with VHSV and remove them before export. Fish sub-clinically infected or carrier fish would not be identified through visual inspection.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the annual likelihood of entry of VHSV was estimated to be **very low**.

11.3.2 Exposure assessment

The key points considered relevant when conducting the direct exposure assessment for VHSV were that:

- VHSV can be transmitted horizontally either by direct contact with diseased fish or indirect contact via contaminated water and equipment. VHSV has been detected in reproductive material and transmission between broodstock and progeny occurs, although true vertical transmission has not been demonstrated.
- VHSV can survive outside the host for a period with survival decreasing with an increase in water temperature.
- VHSV typically infects fish at water temperatures of 1–12°C with virulence greatly reduced at or above 20°C ((Jorgensen 1973a) cited in (Meyers & Winton 1995))(Jorgensen 1982b; Smail 1999).
- VHSV would be expected to be present in sufficient loads in imported fish to cause infection in susceptible species if exposed.
- Species susceptible to VHSV infection are present in Australia include salmonids, *Anguilla* species (eels), *Clupea* species (herrings), *Hoplobrotula armata* (armoured cusk), *Mugil cephalus* (flathead grey mullet), *Seriola dumerili* (greater amberjack), *Scomber* species (mackerel) and sardines.
- Because of the holding conditions in aquarium facilities, any ornamental susceptible species grown with, or sharing the same water as infected imported fish, will likely be exposed to VHSV, however, noting the water temperatures in marine aquariums may generally be expected to be greater than 20°C it is unlikely that infection would occur. Additionally, natural infection has only been detected in wrasse used as cleaner fish in aquaculture setting, these fish are unlikely to be kept as ornamental fish.
- Farmed foodfish in Australia are known to be susceptible to VHSV (e.g. salmonids). However, it is unlikely farmed fish would be in the vicinity of the shore where imported live marine ornamental fish would be released or that they could make their way to a fish cage. Feeding imported marine ornamental fish to farmed foodfish broodstock is assumed to be unlikely because of their high

cost and the large volume and specific nutritional profile that farmed foodfish broodstock require.

- Introduction into the wild may occur by direct release of imported marine ornamental fish or their associated waste/water into natural waters. This would be a direct pathway to wild susceptible species. However, the survival of marine ornamental fish released into the wild is expected to be reduced because of their environmental sensitivity.
- VHSV has a wide host range present in Australian waters that could be infected. However, wild susceptible species would likely be more scattered compared to the dense populations found in aquaculture, which would reduce opportunities for transmission. In addition, the water temperature range required for VHSV infection is present in only a very limited geographical area around Australia (inhibiting not only survival of the ornamental fish, but also VHSV), which impacts the likelihood of exposure of susceptible animals in Australia to viable VHSV.

Conclusion
Based on this information and using the qualitative likelihood descriptors in Table 3, the partial likelihood of exposure of each exposure group to VHSV was estimated to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish—**Extremely low**.
- Wild fish—**Very low**.

11.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to VHSV was determined by combining the likelihood of entry and the partial likelihood of exposure using the matrix in Figure 3 and was found to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish—**Extremely low**.
- Wild fish—**Extremely low**.

11.3.4 Consequence assessment

Partial likelihood of establishment and spread (PLES)
The key points considered relevant when determining the partial likelihood of establishment and spread for VHSV were that:

- VHSV can be transmitted horizontally either by direct contact with diseased fish or indirect contact via contaminated water and equipment. Transmission between broodstock and progeny occurs.
- VHSV can survive outside the host for a period with survival decreasing with an increase in water temperature.
- It is expected that susceptible species in contact with VHSV-infected fish would receive an infectious dose.
- Fish that survive VHSV infections may become carriers and sources of the virus.
- Species susceptible to VHSV infection are present in Australia including salmonids, eels, herrings, armoured cusk, flathead grey mullet, greater amberjack, sardines, and mackerels.

- VHSV typically infects fish at water temperatures of 1–12°C with virulence greatly reduced at or above 20°C ((Jorgensen 1973a) cited in (Meyers & Winton 1995))(Jorgensen 1982b; Smail 1999).
- There are no available treatments for VHSV infection.
- VHSV could establish in ornamental susceptible species. This is due to the stressors associated with aquariums. For example, the higher density of susceptible animals and the holding conditions.
- Fish could be moved to other ornamental facilities in Australia. It is expected that VHSV could establish in these facilities if present in the fish being translocated.
- Each state and territory have translocation protocols for aquaculture animals, which typically includes consideration of VHSV, which would reduce the likelihood of spread between farmed foodfish populations. Though the movement of ornamental fish between hobbyists and retail premises is difficult to monitor and not necessarily captured under all state and territory legislations
- If VHSV were to establish in ornamental facilities supplying a significant part of the hobby sector or many breeders, it could spread widely within this exposure group.
- It is unlikely that VHSV will spread from aquarium industry to farmed foodfish industry due to the lack of exposure pathways and biosecurity practices expected to be in facilities which farm susceptible species.
- It is unlikely that VHSV will spread from an Australian ornamental facility to wild populations via released or escaped fish, given their environmental sensitivity and the anticipated propagule pressure required to ensure establishment of a population.
- Farmed foodfish in Australia are known to be susceptible to VHSV (e.g. salmonids).
- If VHSV were to establish in farmed foodfish populations, it could spread to wild populations through release of water from farms into natural waters. VHSV can survive for extended periods in the environment at low water temperatures. The spread would be moderated by dilution effects and implementation of biosecurity measures. Spread from cage operated farmed foodfish to wild fish would be even more likely due to the sharing of water.
- The use of infected fish such as sardines as feed or bait is another pathway for VHSV spread from farms to wild populations or neighbouring farms.
- Spread of VHSV from foodfish facilities to the aquarium industry is unlikely given the closed systems and lack of exposure pathways between the two exposure groups.
- If one or more index cases of VHSV were to occur in the wild, establishment and spread would be more likely than in aquarium facilities due to the wide host range of VHSV and its transmission via water. The ability of fish to be subclinically infected with VHSV and to remain carriers after surviving an infection would also aid its spread.
- If VHSV were to establish in the wild, it may spread to cage operated farmed foodfish.
- If VHSV were to establish in the wild, especially in waters around aquaculture facilities, it may spread to facilities through water intake. In the absence of effective biosecurity measures, wild infected fish may be transferred into the farms through the inlet water channels.

- Spread of VHSV from the wild to the aquarium industry may occur in cases of subclinically affected species being collected from the wild for sale by the aquarium industry, but any clinically affected fish are unlikely to be harvested from the wild.

Conclusion
Based on these considerations and using the descriptors in Table 3, the partial likelihood of establishment and spread of VHSV in each exposure group for the outbreak scenario was estimated to be:

- Australian ornamental fish—**Extremely low**.
- Farmed foodfish—**Moderate**.
- Wild fish—**Moderate**.

Determining adverse impacts resulting from the outbreak scenario
The factors considered relevant when determining the adverse impacts resulting from establishment and spread of VHSV were that:

Direct effects

The effect on the life or health (including production effects) of susceptible animals and fish

- VHSV causes high mortalities of both farmed and wild fish in freshwater and marine environments. Production and productivity losses due to VHSV would be significant for the Australian salmonid industry with aquaculture production valued at approximately A$1.15 billion in 2021–22 (Tuynman et al. 2023).
- Marine ornamental species other than wrasse may be susceptible to VHSV. The marine ornamental industry would be significantly affected by an outbreak of VHSV if it caused morbidity and mortality.
- VHSV may impact wild fisheries in Australia. There are reports of VHSV in wild fish and associated mortalities and declines in catch rates.
- Based on the impacts of VHSV infection overseas, VHSV establishment and spread in Australia would be expected to cause significant impacts at the national level on the life or health of susceptible species.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There is a wide host range for VHSV in Australia.
- Infection with VHSV has been reported in wild fish populations overseas.
- The direct impact of VHSV establishment and spread on the living environment is expected to be minor at the state or territory level.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Infection with VHSV is listed as a notifiable disease by WOAH and is included on Australia's *National list of reportable diseases of aquatic animals*. States and territories would be required to report on the occurrence of VHSV.

- If VHSV was confirmed at a facility or farm, then an attempt at eradication would be undertaken. The cost of an eradication attempt in affected salmonid farms would be significant for the industry.
- To demonstrate that eradication is successful, there would need to be a surveillance exercise over at least two years to confirm freedom, at considerable cost.
- If VHSV was confirmed in the environment, the inherent difficulties for the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating VHSV from wild populations is unlikely to be undertaken.
- If a movement restriction area were put in place for an outbreak of VHSV, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- Eradication and control of VHSV is expected to cause minor impacts at the national level.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- Movement restriction orders, if put in place, would have indirect impacts on other industries such as seafood suppliers, commercial wild catch fisheries and bait fisheries due to the host range of VHSV.
- Industries supplying inputs into the affected regions may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- VHSV-infected fish may show clinical signs which would affect their marketability.
- VHSV establishment and spread would likely have a minor impact at the state or territory level on domestic trade.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- Infection with VHSV is a WOAH-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to VHSV.
- If VHSV were to become established, Australia could use zoning to maintain access to international markets for live susceptible species and non-viable product.
- The impacts of VHSV establishment and spread on international trade are likely to be minor at the state or territory level.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- VHSV has a wide host range and has been reported in wild fish.
- There are no species listed as endangered in Australia that are related to species known to be susceptible to VHSV.
- A *Epinephelus* species is susceptible to VHSV. If VHSV were to cause disease in *Epinephelus daemelii* (black rock cod), it could have an impact on the survival of this already vulnerable species.

- A conservative approach has been adopted in light of the susceptibility of native species.
- The impacts of VHSV establishment and spread on environmental biodiversity is likely to be minor at the district or region level.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Fish susceptible to VHSV are recreationally fished in Australia and could be affected by mortalities and movement restriction areas put in place which may impact on social amenity.
- In local areas where aquaculture is a major industry, an VHSV outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of VHSV establishment and spread are expected to be minor at the district or region level.

Table 22 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of VHSV. The individual impact scores were combined using the rules in Table 6 to estimate the overall impact (refer section Determining impacts for detailed methodology).

Table 22 Overall impact of establishment and spread of VHSV for the outbreak scenario

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	National	Significant	F
	The environment (native animals/plants, and non-living environment)	State or territory	Minor	D
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	National	Minor	E
	Economic (domestic trade effects and impact on other associated industries)	State or territory	Minor	D
	Economic (international trade effects)	State or territory	Minor	D
	Environment (biodiversity, endangered species and the integrity of ecosystems)	District or region	Minor	C
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	District or region	Minor	C

Conclusion
The overall impact of establishment and spread of VHSV was estimated to be **high**.

11.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for VHSV in each exposure group was determined by combining the partial likelihoods of establishment and spread with the overall impact (using the matrix in Figure 6) and found to be:

- Australian ornamental fish—**Very low**.
- Farmed foodfish—**High**.
- Wild fish—**High**.

11.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of VHSV for each exposure group was determined by combining the partial annual likelihood of entry and exposure with the corresponding likely consequences using the matrix in Figure 7 and found to be:

- Australian ornamental fish— **Negligible**.
- Farmed foodfish— **Very low**.
- Wild fish—**Very low**.

11.3.7 Estimation of overall annual risk

The overall annual risk was estimated by combining the partial annual risk for each exposure group using the rules in Table 7.

The overall annual risk associated with VHSV was found to be **very low**.

Therefore, as the overall annual risk achieves Australia's ALOP, specific biosecurity measures are not considered necessary for this hazard.

12 Proposed biosecurity measures for importation of live marine ornamental fish

The proposed import conditions for live marine ornamental fish imported to Australia are provided in this chapter.

Those seeking to propose alternative biosecurity measures to those presented, should provide a submission to the department for consideration. Such proposals should include supporting scientific data that explain the extent to which the alternative measures would achieve Australia's appropriate level of protection (ALOP).

The final import conditions will be developed based on the proposed biosecurity measures and published on the Australian Biosecurity Import Conditions (BICON) website.

12.1 General requirements

Australia's certification requirements for live marine ornamental fish align with international requirements (refer Chapter 5.1 and 5.2 of the World Organisation for Animal Health (WOAH) Aquatic animal health code (WOAH Code)) and incorporate Australia's import conditions.

In line with certification standards and responsibilities defined in the WOAH Code, the department considers attestations by a country's Competent Authority to provide the most reliable document-level assurance attainable for each country.

Each consignment of live marine ornamental fish must:

- only be exported from a department approved country
- be accompanied by an animal health certificate from the Competent Authority of a department approved country, attesting to the health of the fish in the consignment and the health status of the source population.

12.2 Documentation requirements

Prior to the importation of live marine ornamental fish into Australia, a valid import permit issued by the department is required. Import permits are granted for a one year period, starting from the date of issue. Importers may use the permit to import unlimited numbers of fish during this period (subject to meeting the conditions of the import permit). It is the importer's responsibility to ensure that they have a current permit prior to the import of fish.

Applicants are required to identify that they have access to an approved arrangement site (AA site) for the post-arrival quarantine of aquarium fish (AA for aquarium fish) before a permit application will be approved. AA sites will only be approved, as a place for the isolation of live fish, when they meet the department's standards. An application form for approval of an AA site may be obtained from the department.

12.3 Pre-export requirements

Live marine ornamental fish must only be imported from department approved countries. Animal Quarantine Policy Memorandum 1999/62 provides guidelines for the approval of countries to export animals and their products, including ornamental fish, to Australia.

The Competent Authority of the approved exporting country should ensure that the following criteria are met when approving the exporter and the premises for the export of live marine ornamental fish to Australia:

- The exporting country must have a system in place for the approval of live marine ornamental fish exporters to ensure that such exporters maintain standards required for export of live marine ornamental fish to Australia.
- The Competent Authority of the exporting country must have the authority to suspend or withdraw export certification or approval of an exporter at any time if the requirements are not being met.
- Approval of exporters must be undertaken by a responsible official of the Competent Authority of the exporting country whose duties relate to fish health and who has knowledge of the export operations.

Before approving premises for export of live marine ornamental fish to Australia, a Competent Authority must have in place a system that ensures:

- That the fish being exported to Australia are not sourced from an area associated with any significant infectious fish disease or pests, nor from an area within 5 kilometres of foodfish (fish farmed for human consumption including recreational fishing) aquaculture operations.
- That the fish collection and holding (if applicable) operations do not come into contact with water, equipment or fish associated with farmed foodfish (fish farmed for human consumption including recreational fishing).
- That the fish originate from a facility that is under the supervision of the recognised Competent Authority.
- The competence and integrity of the exporter.
- That the exporter is aware of the conditions which apply to the export of live marine ornamental fish to Australia, including the species permitted for export to Australia at the time of export, and understands the restrictions which apply to such transactions.

All live *Pterapogon* spp. and *Platax* spp. fish present in the consignment must originate from a country, zone or compartment determined by the Competent Authority to be free from megalocytiviruses consistent with the procedures described in Appendix E, or, batch tested prior to export by the Competent Authority and found negative for megalocytiviruses consistent with definitions and testing methodology described in Appendix E.

12.4 Certification

12.4.1 Live marine ornamental finfish exported to Australia (other than *Pterapogon* spp. and *Platax* spp.)

The certifying official must certify that:

1) Only fish on the List of permitted live marine fish suitable for import are included in this consignment and are documented on the attached invoice.

2) The fish were exported from premises approved to export live marine ornamental fish to Australia by a Department of Agriculture, Fisheries and Forestry recognised Competent Authority of the exporting country.

3) The fish in the consignment have been inspected within seven (7) days prior to export and show no clinical signs of infectious disease or pests.

4) The fish are not sourced from a population associated with any significant infectious disease or pests and there have not been any outbreaks of infectious fish disease or pests in the areas from which the fish have been collected during the 6 months prior to collection.

5) The fish have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing).

6) Wild-caught fish were sourced from an area at least five kilometres from any aquaculture facility farming or processing fish for human consumption.

7) Adequate quarantine safeguards are in place to maintain the health status of the certified fish until export. The fish are effectively isolated in holding systems that prevent infection by direct contact with other fish or indirect contact via water, equipment or any other means.

8) The fish in the consignment have not come into contact with water, waste or equipment in common with *Pterapogon* spp. and *Platax* spp..

12.4.2 Live ornamental *Pterapogon* spp. and *Platax* spp. exported to Australia

For *Pterapogon* spp. and *Platax* spp. the Competent Authority in the exporting country must certify that:

1) Each batch of *Pterapogon* spp. and *Platax* spp. in the consignment has been either:

   a) Tested by the Competent Authority and found negative for megalocytiviruses consistent with definitions and testing methodology described by the Department of Agriculture, Fisheries and Forestry in the '*Additional health certification criteria and procedures for Pterapogon* spp. and *Platax* spp *exported to Australia*'. OR,

   b) Sourced from a country, zone or export premises determined by the Competent Authority to be free from megalocytiviruses consistent with the procedures described by the Department of Agriculture, Fisheries and Forestry in the '*Additional health certification criteria and procedures for Pterapogon* spp. and *Platax* spp. *exported to Australia*'.

2) The fish were exported from premises approved to export live marine ornamental fish to Australia by a Department of Agriculture, Fisheries and Forestry recognised Competent Authority of the exporting country.

3) The *Pterapogon* spp. and *Platax* spp. in the consignment have been inspected within seven (7) days prior to export and show no clinical signs of infectious disease or pests.

4) The *Pterapogon* spp. and *Platax* spp. are not sourced from a population associated with any significant infectious disease or pests and there have not been any outbreaks of infectious fish

disease or pests in the areas from which the fish have been collected during the 6 months prior to collection.

5) The *Pterapogon* spp. and *Platax* spp. have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing).

6) Wild-caught *Pterapogon* spp. and *Platax* spp. were sourced from an area at least five kilometres from any aquaculture facility farming or processing fish for human consumption.

7) Adequate quarantine safeguards are in place to maintain the health status of the certified *Pterapogon* spp. and *Platax* spp. until export. The fish are effectively isolated in holding systems that prevent infection by direct contact with other fish or indirect contact via water, equipment or any other means.

12.5 Transport

All fish in the consignment must be packaged in accordance with International Air Transport Association (IATA) Live Animal Regulations.

All fish in the consignment must be packaged in leak-proof bags with each bag containing only one species. The bag must be colourless and sufficiently transparent to enable proper inspection and identification of the fish and must not contain any extraneous matter, unapproved plant material, pests or unauthorised species of fish. The use of outer bags of opaque materials or half-black bags to provide a dark shipping environment is acceptable provided the contents of the bag can be properly inspected to the department's satisfaction.

The inclusion of inert material such as zeolite, activated carbon, shredded plastic or dried terrestrial plants is permitted provided the contents of the bag can be properly inspected to the department's satisfaction and the material is disinfected or disposed of as directed by the department.

The bags must be placed within polystyrene boxes or cartons fitted with a plastic lining. Each box or carton must be clearly identified as a part of a consignment and be individually identified.

The consignment must be accompanied by documents that include the identification number of each box or carton, and the scientific name and number of the contained fish. It is recommended that the common names of the fish also be included on the papers.

Each bag must be stocked at a density that will allow inspection and must not be overcrowded. When packed for export, fish must be placed in clean water. The use of a pH indicator in the water is permissible, provided it does not interfere with inspection.

12.6 On-arrival inspections

All shipments of live marine ornamental fish will be inspected by the department on arrival to ensure that:

- they are healthy and not overtly diseased
- the health certification and invoice are in order
- they are an approved species listed on the List of permitted live marine fish suitable for import
- they do not contain non-permitted material or material of biosecurity concern.

Fish not meeting these criteria and non-permitted material will be exported or disposed of at the importer's expense.

Consignments that do not meet the department's import conditions will remain in biosecurity control and be exported or disposed of. Any fish species not listed on the List of permitted live marine fish suitable for import must be exported or disposed of, while non-permitted material or material of biosecurity concern will be forfeited and disposed of.

Each consignment must only include fish that are sufficiently mature to permit accurate identification. Fish that cannot be identified will be exported or disposed of.

12.7 Post-arrival quarantine

Live marine ornamental fish that have been inspected by the department on arrival and found to satisfy all import conditions will be moved directly to the AA site named on the import permit for a minimum of 7 days. Any significant event occurring during transport of the fish to the AA site must be reported to the department within 2 hours of the event.

Based on fish species, country of origin, historical factors or any other relevant information, the department may test samples of imported fish during quarantine to determine their health status. The cost of testing will be borne by the person in charge of the goods.

In the event of any imported fish showing clinical signs of an infectious disease, or producing a positive result to any tests indicating the presence of an infectious disease agent or pest, the department may cause any or all of the fish in the AA site to be either detained in quarantine for further observation, tested and treated, or to be disposed of.

At the end of the quarantine period, the fish will be inspected by the department and must be found free from clinical signs of pest and disease before they will be released from biosecurity control.

12.8 Review of processes

The department reserves the right to review the import policy. The department may review the policy after the first year of trade:

- when there is reason to believe that the disease or sanitary status in exporting countries has changed
- when there are large changes in the volume of live marine ornamental fish imported
- when Australian industries that may be impacted by disease agents potentially imported in live marine ornamental fish evolve such that the biosecurity risk is greater.

Appendix A List of permitted live marine ornamental fish suitable for import

Table 23 contains the department's List of permitted live marine ornamental fish suitable for import sourced from all countries and whether they are reported to have been captive bred (CORAL Magazine 2019).

Table 23 List of permitted live marine ornamental fish suitable for import and whether they are reported to have been successfully captive bred

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
Surgeonfish, Tang, Unicorn fish	*Acanthurus* spp.	Yes
	Ctenochaetus spp.	No
	Naso spp.	Yes
	Paracanthurus spp.	Yes
	Prionurus spp.	No
	Zebrasoma spp.	Yes
Flashlight fish, Lanterneye fish	*Anomalops* spp.	No
	Kryptophanaron spp.	No
	Parmops spp.	No
	Photoblepharon spp.	No
	Phthanophaneron spp.	No
	Protoblepharon spp.	No
Cardinal fishes	*Apogon* spp.	Yes
	Apogonichthyoides spp.	Yes
	Apogonichtys spp.	No
	Archamia spp.	No
	Astrapogon spp.	No
	Cercamia spp.	No
	Cheilodipterus spp.	Yes
	Coranthus spp.	No
	Foa spp.	No
	Fowleria spp.	Yes
	Glossamia spp.	No
	Gymnapogon spp.	No
	Holapogon spp.	No
	Jaydia spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Lachneratus spp.	No
	Neamia spp.	No
	Nectamia spp.	Yes
	Ostorhinchus spp.	Yes
	Paxton spp.	No
	Phaeoptyx spp.	No
	Pseudamia spp.	No
	Pseudamiops spp.	No
	Pterapogon spp.	Yes
	Rhabdamia spp.	No
	Siphamia spp.	No
	Sphaeramia spp.	Yes
	Vincentia spp.	No
	Zoramia spp.	Yes
Triggerfish	*Abalistes* spp.	No
	Balistapus spp.	No
	Balistes spp.	Yes
	Balistoides spp.	Yes
	Canthidermis spp.	No
	Melichthys spp.	No
	Odonus spp.	No
	Pseudobalistes spp.	No
	Rhinecanthus spp.	No
	Sufflamen spp.	No
	Xanthichthys spp.	Yes
	Xenobalistes spp.	No
Viviparous brotulas	*Acarobythites* spp.	No
	Alionematichthys spp.	No
	Anacanthobythites spp.	No
	Beaglichthys spp.	No
	Bellottia spp.	No
	Bidenichthys spp.	No
	Brosmodorsalis spp.	No
	Brosmolus spp.	No
	Brosmophyciops spp.	No
	Brosmophycis spp.	No
	Brotulinella spp.	No
	Bythites spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Calamopteryx spp.	No
	Cataetyx spp.	No
	Dactylosurculus spp.	No
	Dermatopsis spp.	No
	Dermatopsoides spp.	No
	Diancistrus spp.	No
	Didymothallus spp.	No
	Dinematichthys spp.	No
	Diplacanthopoma spp.	No
	Dipulus spp.	No
	Eusurculus spp.	No
	Fiordichthys spp.	No
	Grammonus spp.	No
	Gunterichthys spp.	No
	Hastatobythites spp.	No
	Hephthocara spp.	No
	Lapitaichthys spp.	No
	Lucifuga spp.	No
	Majungaichthys spp.	No
	Mascarenichthys spp.	No
	Melodichthys spp.	No
	Microbrotula spp.	No
	Monothrix spp.	No
	Nielsenichthys spp.	No
	Ogilbia spp.	No
	Ogilbichthys spp.	No
	Paradiancistrus spp.	No
	Porocephalichthys spp.	No
	Pseudogilbia spp.	No
	Pseudonus spp.	No
	Saccogaster spp.	No
	Stygnobrotula spp.	Yes
	Thalassobathia spp.	No
	Thermichthys spp.	No
	Timorichthys spp.	No
	Tuamotuichthys spp.	No
	Typhliasina spp.	No
	Ungusurculus spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Zephyrichthys spp.	No
Dragonets	*Anaora* spp.	No
	Bathycallionymus spp.	No
	Callionymus spp.	Yes
	Calliurichthys spp.	No
	Dactylopus spp.	No
	Diplogrammus spp.	No
	Draculo spp.	No
	Eleutherochir spp.	No
	Eocallionymus spp.	No
	Foetorepus spp.	No
	Minysynchiropus spp.	No
	Neosynchiropus spp.	No
	Paracallionymus spp.	No
	Protogrammus spp.	No
	Pseudocalliurichthys spp.	No
	Repomucenus spp.	No
	Synchiropus spp.	Yes
	Tonlespia spp.	No
Pearlfish	*Carapus* spp.	No
	Echiodon spp.	No
	Encheliophis spp.	No
	Eurypleuron spp.	No
	Onuxodon spp.	No
	Pyramodon spp.	No
	Snyderidia spp.	No
	Tetragondacnus spp.	No
Snipefish, Shrimpfish	*Aeoliscus* spp.	Yes
	Centriscops spp.	No
	Centriscus spp.	No
	Macroramphosus spp.	No
	Notopogon spp.	No
Butterfly fish	*Amphichaetodon* spp.	No
	Chaetodon spp.	Yes
	Chelmon spp.	No
	Chelmonops spp.	No
	Coradion spp.	No
	Forcipiger spp.	Yes

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Hemitaurichthys spp.	No
	Heniochus spp.	No
	Johnrandallia spp.	No
	Parachaetodon spp.	Yes
	Prognathodes spp.	No
	Roa spp.	No
Hawk fish	*Amblycirrhitus* spp.	No
	Cirrhitichthys spp.	No
	Cirrhitops spp.	No
	Cirrhitus spp.	No
	Cristacirrhitus spp.	No
	Cyprinocirrhites spp.	No
	Isocirrhitus spp.	No
	Itycirrhitus spp.	No
	Neocirrhitus spp.	No
	Notocirrhitus spp.	No
	Oxycirrhites spp.	No
	Paracirrhites spp.	No
Batfish, Spadefish, Scats	*Chaetodipterus* spp.	Yes
	Ephippus spp.	No
	Parapsettus spp.	No
	Platax spp.	Yes
	Proteracanthus spp.	No
	Rhinoprenes spp.	No
	Tripterodon spp.	No
	Zabidius spp.	No
Basslets	*Gramma* spp.	Yes
	Lipogramma spp.	Yes
Squirrelfish, Soldierfish	*Corniger* spp.	No
	Holocentrus spp.	No
	Myripristis spp.	No
	Neoniphon spp.	No
	Ostichthys spp.	No
	Plectrypops spp.	No
	Pristilepis spp.	No
	Sargocentron spp.	No
Wrasses	*Acantholabrus* spp.	No
	Achoerodus spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Ammolabrus spp.	No
	Anampses spp.	No
	Anchichoerops spp.	No
	Austrolabrus spp.	No
	Bodianus spp.	Yes
	Centrolabrus spp.	No
	Cheilinus spp. (excluding *Ceilinus undulatus*)	Yes
	Cheilio spp.	No
	Choerodon spp.	No
	Cirrhilabrus spp.	No
	Clepticus spp.	No
	Conniella spp.	No
	Coris spp.	No
	Ctenolabrus spp.	No
	Cymolutes spp.	No
	Decodon spp.	No
	Diproctacanthus spp.	No
	Doratonotus spp.	No
	Dotalabrus spp.	No
	Epibulus spp.	No
	Eupetrichthys spp.	No
	Frontilabrus spp.	No
	Gomphosus spp.	No
	Halichoeres spp.	Yes
	Hemigymnus spp.	No
	Hologymnosus spp.	No
	Iniistius spp.	No
	Labrichthys spp.	No
	Labroides spp.	Yes
	Labropsis spp.	No
	Labrus spp.	No
	Lachnolaimus spp.	Yes
	Lappanella spp.	No
	Larabicus spp.	No
	Leptojulis spp.	No
	Macropharyngodon spp.	No
	Malapterus spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Minilabrus spp.	No
	Nelabrichthys spp.	No
	Notolabrus spp.	No
	Novaculichthys spp.	No
	Novaculoides spp.	No
	Ophthalmolepis spp.	No
	Oxycheilinus spp.	No
	Oxyjulis spp.	No
	Paracheilinus spp.	No
	Parajulis spp.	Yes
	Pictilabrus spp.	No
	Polylepion spp.	No
	Pseudocheilinops spp.	No
	Pseudocheilinus spp.	No
	Pseudocoris spp.	No
	Pseudodax spp.	No
	Pseudojuloides spp.	No
	Pseudolabrus spp.	No
	Pteragogus spp.	No
	Semicossyphus spp.	No
	Stethojulis spp.	No
	Suezichthys spp.	No
	Symphodus spp.	No
	Tautoga spp.	No
	Tautogolabrus spp.	No
	Terelabrus spp.	No
	Thalassoma spp.	No
	Wetmorella spp.	No
	Xenojulis spp.	No
	Xiphocheilus spp.	No
	Xyrichtys spp.	No
Blanquillos, Tilefish	*Branchiostegus* spp.	No
	Caulolatilus spp.	No
	Hoplolatilus spp.	No
	Lopholatilus spp.	No
	Malacanthus spp.	No
Filefish	*Acanthaluteres* spp.	No
	Acreichthys spp.	Yes

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Aluterus spp.	No
	Amanses spp.	No
	Anacanthus spp.	No
	Brachaluteres spp.	No
	Cantherhines spp.	No
	Cantheschenia spp.	No
	Chaetodermis spp.	No
	Colurodontis spp.	No
	Enigmacanthus spp.	No
	Eubalichthys spp.	No
	Lalmohania spp.	No
	Meuschenia spp.	No
	Monacanthus spp.	No
	*Nelusett*a spp.	No
	Oxymonacanthus spp.	Yes
	Paraluteres spp.	No
	Paramonacanthus spp.	No
	Pervagor spp.	No
	Pseudalutarius spp.	No
	Pseudomonacanthus spp.	No
	Rudarius spp.	Yes
	Scobinichthys spp.	No
	Stephanolepis spp.	Yes
	Thamnaconus spp.	No
Pinecone fish	*Cleidopus* spp.	No
	Monocentris spp.	No
Goatfish	*Mulloidichthys* spp.	No
	Mullus spp.	No
	Parupeneus spp.	No
	Pseudupeneus spp.	No
	Upeneichthys spp.	No
	Upeneus spp.	No
Moray eels	*Anarchias* spp.	No
	Channomuraena spp.	No
	Cirrimaxilla spp.	No
	Diaphenchelys spp.	No
	Echidna spp.	No
	Enchelycore spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Enchelynassa spp.	No
	Gymnomuraena spp.	No
	*Gymnothora*x spp.	No
	Monopenchelys spp.	No
	Muraena spp.	No
	Pseudechidna spp.	No
	Rhinomuraena spp.	No
	Scuticaria spp.	No
	Strophidon spp.	No
	Uropterygius spp.	No
Box fish	*Acanthostracion* spp.	Yes
	Lactophrys spp.	No
	Lactoria spp.	No
	Ostracion spp.	No
	Paracanthostracion spp.	No
	Rhinesomus spp.	No
	Rhynchostracion spp.	No
	Tetrosomus spp.	No
Seamoths	*Eurypegasus* spp.	No
	Pegasus spp.	No
Sweepers	*Parapriacanthus* spp.	No
	Pempheris spp.	Yes
Convict blennies	*Pholidichthys* spp.	Yes
Weevers, Sandperches	*Kochichthys* spp.	No
	Parapercis spp.	No
	Pinguipes spp.	No
	Prolatilus spp.	No
	Pseudopercis spp.	No
	Ryukyupercis spp.	No
	Simipercis spp.	No
Roundheads	*Acanthoclinus* spp.	No
	Acanthoplesiops spp.	No
	Assessor spp.	No
	Beliops spp.	No
	Belonepterygion spp.	No
	Calloplesiops spp.	Yes
	Fraudella spp.	No
	Notograptus spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Paraplesiops spp.	No
	Plesiops spp.	Yes
	Steeneichthys spp.	No
	Trachinops spp.	Yes
Angelfish	*Apolemichthys* spp.	Yes
	Centropyge spp.	Yes
	Chaetodontoplus spp.	Yes
	Genicanthus spp.	Yes
	Holacanthus spp.	Yes
	Pomacanthus spp.	Yes
	Pygoplites spp.	Yes
Damselfish, Clownfish	*Abudefduf* spp.	Yes
	Acanthochromis spp.	Yes
	Altrichthys spp.	No
	Amblyglyphidodon spp.	Yes
	Amblypomacentrus spp.	No
	Amphiprion spp.	Yes
	Azurina spp.	No
	Cheiloprion spp.	No
	Chromis spp.	Yes
	Chrysiptera spp.	Yes
	Dascyllus spp.	Yes
	Dischistodus spp.	No
	Hemiglyphidodon spp.	No
	Hypsypops spp.	Yes
	Lepidozygus spp.	No
	Mecaenichthys spp.	No
	Microspathodon spp.	Yes
	Neoglyphidodon spp.	Yes
	Neopomacentrus spp.	Yes
	Nexilosus spp.	No
	Parma spp.	No
	Plectroglyphidodon spp.	No
	Pomacentrus spp.	Yes
	Pomachromis spp.	No
	Premnas spp.	Yes
	Pristotis spp.	No
	Similiparma spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Stegastes spp.	No
	Teixeirichthys spp.	No
Big eyes, Catalufas	*Cookeolus* spp.	No
	Heteropriacanthus spp.	No
	Priacanthus spp.	No
	Pristigenys spp.	No
Dottybacks	*Amsichthys* spp.	No
	Anisochromis spp.	No
	Assiculoides spp.	No
	Assiculus spp.	No
	Blennodesmus spp.	No
	Chlidichthys spp.	No
	Congrogadus spp.	Yes
	Cypho spp.	Yes
	Halidesmus spp.	No
	Halimuraena spp.	No
	Halimuraenoides spp.	No
	Haliophis spp.	No
	Labracinus spp.	No
	Lubbockichthys spp.	No
	Manonichthys spp.	Yes
	Natalichthys spp.	No
	Ogilbyina spp.	Yes
	Oxycercichthys spp.	Yes
	Pectinochromis spp.	No
	Pholidochromis spp.	No
	Pictichromis spp.	Yes
	Pseudochromis spp.	Yes
	Pseudoplesiops spp.	Yes
	Rusichthys spp.	No
Parrotfish	*Bolbometopon* spp.	No
	Calotomus spp.	No
	Cetoscarus spp.	No
	Chlorurus spp.	No
	Cryptotomus spp.	No
	Hipposcarus spp.	No
	Leptoscarus spp.	No
	Nicholsina spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Scarus spp.	No
	Sparisoma spp.	No
Pipefish, Seahorses	*Acentronura* spp.	No
	Amphelikturus spp.	No
	Anarchopterus spp.	No
	Apterygocampus spp.	No
	Bhanotia spp.	No
	Bryx spp.	No
	Bulbonaricus spp.	No
	Campichthys spp.	No
	Choeroichthys spp.	No
	Corythoichthys spp.	No
	Cosmocampus spp.	No
	Doryichthys spp.	No
	Doryrhamphus spp.	Yes
	Dunckerocampus spp.	Yes
	Enneacampus spp.	No
	Entelurus spp.	No
	Festucalex spp.	No
	Filicampus spp.	No
	Halicampus spp.	No
	Haliichthys spp.	Yes
	Heraldia spp.	No
	Hippichthys spp.	No
	Histiogamphelus spp.	No
	Hypselognathus spp.	No
	Ichthyocampus spp.	No
	Idiotropiscis spp.	No
	Kaupus spp.	No
	Kimblaeus spp.	No
	Kyonemichthys spp.	No
	Leptoichthys spp.	No
	Leptonotus spp.	No
	Lissocampus spp.	No
	Maroubra spp.	No
	Micrognathus spp.	No
	Microphis spp.	No
	Minyichthys spp.	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
	Mitotichthys spp.	No
	Nannocampus spp.	No
	Nerophis spp.	No
	Notiocampus spp.	No
	Penetopteryx spp.	No
	Phoxocampus spp.	No
	Phycodurus spp.	No
	Phyllopteryx spp.	Yes
	Pseudophallus spp.	No
	Pugnaso spp.	No
	Siokunichthys spp.	No
	Solegnathus spp.	Yes
	Stigmatopora spp.	No
	Stipecampus spp.	No
	Syngnathoides spp.	Yes
	Syngnathus spp.	Yes
	Trachyrhamphus spp.	No
	Urocampus spp.	No
	Vanacampus spp.	No
Trevally	*Alectis* spp.	No
Rock cods	*Anthias* spp.	No
Sole	*Brachirus* spp.	No
Puffer fish	*Canthigaster* spp.	Yes
Red-streaked blenny, Reticulated blenny	*Cirripectes stigmaticus*	No
Groupers (excluding Barramundi cod)	*Cromileptes* spp. (excluding *Cromileptes altivelis*)	No
Scorpion fish	*Dendrochirus* spp.	No
Axelrod's clown blenny	*Ecsenius axelrodi*	No
Two-colour comb-tooth blenny, Bicolour comb-tooth blenny, Bicolour blenny	*Ecsenius bicolor*	Yes
Red sea mimic blenny	*Ecsenius gravieri*	No
Yellow-eyed comb-tooth blenny	*Ecsenius melarchus*	No
Midas blenny comb-tooth blenny, Lyre-tail comb-tooth, Persian blenny	*Ecsenius midas*	No
Blenny	*Ecsenius pulcher*	No
Gobies	*Gobiodon* spp.	Yes
Zebra bullhead shark	*Heterodontus zebra*	Yes
Black-headed blenny	*Lipophrys nigriceps*	No

Common name(s)	Genus or species permitted import to Australia [a]	Genus or species included on the 2019 Captive bred list (Yes/No) [b]
Jumping cod	*Lobotes* spp.	No
Gobies	*Lythrypnus* spp.	Yes
Snapper	*Macolor* spp.	No
Eyelash fang blenny, Eyelash harptail blenny, Lyretail blenny, Yellow-tail fang blenny, Forktail blenny	*Meiacanthus atrodorsalis*	Yes
Linespot fang blenny, Black-banded blenny, Line-spot harp-tail blenny, Striped poison fang blenny	*Meiacanthus grammistes*	Yes
Canary fang blenny, Oualan forktail blenny	*Meiacanthus oualanensis*	Yes
Gobies	*Nemateleotris* spp.	Yes
Eel-tailed catfish, Striped eel catfish, Lined catfish	*Plotosus lineatus*	Yes
Gobies	*Ptereleotris* spp.	No
Lionfish	*Pterois* spp.	No
Scorpion fish	*Rhinopias* spp.	No
Spine-cheek, Two-lined monocle bream	*Scolopsis bilineata*	No
Rabbit fishes	*Siganus* spp.	Yes
Gobies	Signigobius spp.	Yes
Sailfin Snapper	*Symphorichthys* spp.	No
Blue spotted fantail ray	*Taeniura lymma*	Yes
Blueband glider goby, Bluestreak goby, Golden-head sleeper goby	*Valenciennea strigata*	No
Moorish idol	*Zanclus cornutus*	No

[a] Genus or species are permitted import according to the List of permitted live marine ornamental fish suitable for import (Department of Agriculture 2023b).

[b] One or more species within the genus are reported to have been successfully bred in captivity and are featured on CORAL Magazine's captive-bred marine fish species list for 2019 (CORAL Magazine 2019).

[c] Clarion Angelfish (*Holacanthus clarionensis*) require an additional Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) import permit issued by the Department of Climate Change, Energy, the Environment and Water.

[d] Excluding *Siganus rivulatus* and *Siganus luridus*.

Appendix B Review of Permitted marine species list in relation to potential for import for use in aquaculture for human consumption

Cromileptes altivelis (barramundi cod)

Background

Cromileptes altivelis (barramundi cod) was among the species considered in the *Import risk analysis on live ornamental fish* released in 1999 (Ornamental fish IRA) (AQIS 1999). They were permitted import providing they were sourced from wild populations and were destined for public or home aquaria in Australia.

In 2004 it was determined that advances in the aquaculture industry may have implications for the source and end-use of imported barramundi cod. As a result, Animal Biosecurity Policy Memorandum 2004/18 was published, which suspended the import of live barramundi cod. Barramundi cod were subsequently removed from the department's List of permitted live marine ornamental fish suitable for import (*Permitted marine species list*).

Barramundi cod sourced from aquaculture for human consumption, could be expected to present different risks than wild-caught fish or fish grown for ornamental purposes (where the value of the product is more, and the intended end-use is in an aquaria). The risk factors include crowding, stress, absence of predators and other conditions that favour the multiplication of disease agents, survival of infected hosts and increased prevalence of disease agents. Similarly, the likelihood of exposure of fish in Australia's natural waters to a disease agent via an imported fish will be different if imported for aquaculture grow-out in open or semi-open systems, or as broodstock in hatcheries.

Barramundi cod aquaculture has been steadily increasing since 2004, with large-scale catch and grow out of juveniles becoming increasingly common across Asia (Khasanah et al. 2020). Barramundi cod now represents an important economic product in Asia, becoming the main farmed species in several regions – including the southern coastal areas of China (Chen et al. 2023). Sea cage farms can produce anywhere from 400 to over 4,000 kilograms of barramundi cod per month depending on the scale of the farm, with small farms having less than 10 cages and large farms having close to 50 cages (Afero, Miao & Perez 2010). Cultured grouper only represents 8.6% of the total 52,000 metric tonnes of annual grouper production, the rest being wild-caught (Afero, Miao & Perez 2010). In Australia, barramundi cod are subject to several conservation measures, including being a no-take species in Queensland, and a possession limit species in other states – including Western Australia.

Outcome

The outcomes of this draft risk review are that whilst live marine ornamental fish may be permitted import, they are not permitted import if they have been sourced from populations that are

associated with farmed foodfish. This is to reduce the likelihood of hazards that are often associated with farmed foodfish being spread to the marine ornamental fish being exported to Australia.

Given the apparent popularity of barramundi cod aquaculture for human consumption (foodfish) purposes, and the current restriction on barramundi cod fishing in Australia, the department considers that the decision made in 2004 regarding barramundi cod is still appropriate. The likelihood of the imported barramundi cod being sourced from populations which have been grown for human consumption or that are in close contact with fish cultured for human consumption, in combination with the potential for them to be used for aquaculture in Australia, exceeds Australia's appropriate level of protection (ALOP). Therefore, barramundi cod will not be reinstated on the department's *Permitted marine species list*.

Review of other species

Species of live marine ornamental fish included on the *Permitted marine species list*, other than barramundi cod, are reported to have been successfully captive bred (CORAL Magazine 2019) and that may also be used for food (usually depending on their life-stage) are listed in Table 24.

Table 24 Captive bred marine fish species that are reported to have been used for both human consumption and ornamental purposes and whether they are permitted import into Australia

Family	Scientific name	Common name	Genus or species permitted import to Australia
Batrachoididae	*Opsanus tau*	Oyster toadfish	No
Ephippidae	*Chaetodipterus faber*	Atlantic spadefish	Yes
	Platax batavianus	Humpback batfish	Yes
	Platax orbicularis	Orbicular batfish	Yes
	Platax pinnatus	Dusky batfish	Yes
Lutjanidae	*Lutjanus sebae*	Emperor red snapper	No
Serranidae	*Cromileptes altivelis*	Humpback grouper (barramundi cod)	Refer to: *Cromileptes altivelis* (barramundi cod)
	Epinephelus lanceolatus	Giant grouper	No
	Epinephelus marginatus	Dusky grouper	No
	Plectropomus areolatus	Squaretail coral grouper	No
	Plectropomus leopardus	Leopard coral grouper	No
Siganidae	*Siganus canaliculatus*	White-spotted spinefoot	Yes
	Siganus fuscescens	Mottled spinefoot	Yes
	Siganus guttatus	Orange-spotted spinefoot	Yes
	Siganus lineatus	Golden-lined spinefoot	Yes
	Siganus rivulatus	Marbled spinefoot	No
	Siganus vermiculatus	Vermiculated spinefoot	Yes
Tetraodontidae	*Sphoeroides annulatus*	Bullseye puffer	No

	Sphoeroides maculatus	Northern puffer	No

Following a review of captive bred marine fish species that are reported to have been used for both human consumption and ornamental purposes (Pouil et al. 2020) and which are permitted import into Australia, the department concluded that two genera of live marine ornamental fish on the department's *Permitted marine species list* required the same review and consideration that was given to *C. altivelis*.

Platax species

Background

Platax species (batfish) were among those considered in the Ornamental fish IRA, and they were permitted import providing they were sourced from wild populations and were destined for public or home aquaria in Australia.

Batfish sourced from aquaculture for human consumption, could be expected to present different risks than wild-caught fish or fish grown for ornamental purposes (where the value of the product is more). The risk factors are likely to be crowding, stress, absence of predators and other conditions that favour the multiplication of disease agents, survival of infected hosts and increased prevalence of disease agents. Similarly, the likelihood of exposure of fish in Australia's natural waters to a disease agent via an imported fish will be different if imported for aquaculture grow-out in open or semi-open systems, or as broodstock in hatcheries.

Batfish juveniles are attractive for the ornamental market, with adults being utilised for human consumption in Asian and South Pacific regions (Barros et al. 2011; Leu et al. 2018). Several batfish species, such as *Platax teira* in Asia, and *Platax orbicularis* in French Polynesia, are growing in popularity as aquaculture species (Leu et al. 2018; Reverter et al. 2016). Whilst no exact volumes for production are known, aquaculture practices of this species are limited to semi-intensive and lagoon fish farming.

Outcome

The outcomes of this draft risk review are that whilst live marine ornamental fish may be permitted import, they are not permitted import if they have been sourced from populations that are associated with farmed foodfish.

Given the lack of information known concerning the exact production of batfish as foodfish, it is unlikely that imported batfish will be sourced from these populations. As such, the department does not consider the import of batfish will pose a greater risk than other permitted species of live marine ornamental fish that are not also considered foodfish. The likelihood of imported batfish being sourced from populations which have been grown for human consumption, or that are in close contact with fish cultured for human consumption, in combination with the potential for them to be used for aquaculture in Australia does not exceed Australia's ALOP.

Siganus species

Background

Siganus species (excluding *Siganus rivulatus* and *Siganus luridus*) (rabbitfish) were among those considered in the Ornamental fish IRA, and were permitted import providing they were sourced from wild populations and were destined for public or home aquaria in Australia.

Rabbitfish sourced from aquaculture for human consumption, could be expected to present different risks than wild-caught fish or fish grown for ornamental purposes (where the value of the product is more). The risk factors are likely to be crowding, stress, absence of predators and other conditions that favour the multiplication of disease agents, survival of infected hosts and increased prevalence of disease agents. Similarly, the likelihood of exposure of fish in Australia's natural waters to a disease agent via an imported fish will be different if imported for aquaculture grow-out in open or semi-open systems, or as broodstock in hatcheries.

Rabbitfish are growing in economic value as the become the favoured fish across several regions, particularly Indonesia (Parawansa et al. 2020), Malaysia, and the Philippines (Jaikumar 2012). In the Philippines, rabbitfish represent 560 metric tonnes of the of the total annual fishery production (Jaikumar 2012). Aquaculture of rabbitfish is predominantly non-intensive, limited to wild-caught populations, or populations grown out in brackish ponds or embanked lagoons. However, there is a growing trend of cage culture, particularly for *Siganus canaliculatus* due to them being fast-growing and hardy. Specific details concerning the farmed production volumes of rabbitfish are limited.

The prominent rabbitfish species used as foodfish are *S. canaliculatus, Siganus guttatus, Siganus virgatus, Siganus spinus, Siganus punctatus, Siganus fuscescens, Siganus rivulatus, Siganus randalli* and *Siganus lineatus*. Several of these are regularly imported and are easily obtained in Australia as ornamental fish.

Outcome

The outcomes of this draft risk review are that whilst live marine ornamental fish may be permitted import, they are not permitted import if they have been sourced from populations that are associated with farmed foodfish.

Given the limited farmed production of rabbitfish as foodfish, it is unlikely that imported *Siganus* will be sourced from these populations. As such, the department does not consider the import of *Siganus* will pose a greater risk than other permitted species of marine ornamental fish that are not also considered foodfish. The likelihood of imported rabbitfish being sourced from populations which have been grown for human consumption, or that are in close contact with fish cultured for human consumption, in combination with the potential for them to be used for aquaculture in Australia does not exceed Australia’s ALOP.

Appendix C Species susceptibility lists

Full susceptible species lists for each hazard are presented in this appendix in full.

Aeromonas salmonicida (typical)

All *Salmonidae* species (salmonids) and *Anguillidae* species (eels) are believed to be susceptible to infection with typical *A. salmonicida* through natural exposure (Australian Government Department of Agriculture 2019; Dallaire-Dufresne et al. 2014). Non-salmonid species are also susceptible and it is proposed that few cultured or feral fish are immune (Cipriano & Bullock 2001b). Non-salmonid species which are reported to be susceptible to infection with typical *A. salmonicida* include, but are not limited to those listed in Table 25.

Table 25 Non-salmonid species which are reported to be susceptible to infection with typical *A. salmonicida*

Family	Species	Common name	Reference
Anguillidae	All	Eel	(Australian Government Department of Agriculture 2019; Dallaire-Dufresne et al. 2014)
Acipenseridae	*Acipenser oxyrinchus*	Atlantic sturgeon	(Mohler 2003)
Catostomidae	*Catostomus commersoni*	White sucker	(Ostland, Hicks & Daly 1987)
	Coreius guichenoti	Largemouth bronze gudgeon	(Long et al. 2016)
Cyprinidae	*Carassius auratus*	Goldfish	(Lian et al. 2020)
	Ctenopharyngodon idella	Grass carp	(Long et al. 2016)
	Luxilus cornutus	Common shiner	(Ostland, Hicks & Daly 1987)
Gadidae	*Gadus morhua*	Atlantic cod	(Boily, Malcolm & Johnson 2019)
Labridae	All	Wrasse	(Treasurer & Laidler 1994)
Latidae	*Lates calcarifer*	Barramundi, Asian sea bass	(Mukherjee, Peer Mohamed & Alavandi 1989)
Moronidae	*Dicentrarchus labrax*	European bass	(Fernández-Álvarez et al. 2016)
Percidae	*Perca flavescens*	Yellow perch	(Diamanka et al. 2013)
	Perca fluviatilis	European perch, redfin	(Skrodenyte-Arbaciauskiene et al. 2010)
	Sander lucioperca	Pike-perch	(Schulz et al. 2020)
Petromyzontidae	*Petromyzon mariunus*	Sea lamprey	(Diamanka et al. 2013)
Pleuronectidae	*Hippoglossus hippoglossus*	Atlantic halibut	(Bricknell et al. 1999)
Polydontidae	*Polydon spathula*	Mississippi paddlefish	(Ford, Cipriano & Penniston 1994)
Salmonidae	All	Salmon, trout	(Australian Government Department of Agriculture 2019; Dallaire-Dufresne et al. 2014)
Scophthalmidae	*Scophthalmus maximus*	Turbot	(Farto et al. 2011; Toranzo & Barja 1992)
Serranidae	*Epinephelus coioides*	Orange spot grouper, estuary cod	(Reith et al. 2008)

Sparidae	*Sparus aurata*	Gilthead seabream	(Real et al. 1994)

Enteromyxum leei

Species which are susceptible to infection with *E. leei* include, but are not limited to, those listed in Table 26.

Table 26 Species which are susceptible to infection with *E. leei*

Family	Species	Common name	Reference
Acanthuridae	*Acanthurus leucosternon*	Powder blue tang	(Hyatt et al. 2018)
	Acanthurus nigrofuscus	Lavender tang	(Diamant, Colorni & Ucko 2004)
	Zebrasoma flavescens	Yellow tang	(Hyatt et al. 2018)
Batrachoididae	*Halobatrachus didactylus*	Lusitanian toadfish	(Padrós et al. 2001)
Blenniidae	*Cirripectes filamentosus*	Filamentous blenny	(Diamant, Colorni & Ucko 2004)
	Parablennius spp.	Blennids	(Padrós et al. 2001)
	Salaria pavo	Peacock blenny	(Padrós et al. 2001)
Epinephelidae	*Epinephelus malabaricus*	Malabar grouper	(China et al. 2013)
Gobiidae	*Gobius niger*	Black goby	(Padrós et al. 2001)
Labridae	*Cheilinus undulatus*	Humphead wrasse	(Katharios, Rigos & Divanach 2011)
	Coris aygula	Clown coris	(Hyatt et al. 2018)
	Coris julis	Mediterranean rainbow wrasse	(Padrós et al.)
	Labrus spp.	Wrasse	(Padrós et al. 2001)
	Symphodus sp.	Wrasse	(Padrós et al. 2001)
	Thalassoma pavo	Ornate wrasse	(Padrós et al. 2001)
Lutjanidae	*Lutjanus Kasmira*	Bluestripe snapper	(Hyatt et al. 2018)
Molidae	*Mola mola*	Ocean sunfish	(Padrós et al. 2001)
Moronidae	*Dicentrarchus labrax*	European sea bass	(Sitjà-Bobadilla et al. 2007)
Mugilidae	*Chelon auratus*	Golden grey mullet	(Diamant 1998b)
	Chelon ramada	Thinlip grey mullet	(Diamant 1998b)
	Chelon saliens	Leaping mulet	(Diamant 1998b)
Mullidae	*Mulloidichthys vanicolensis*	Yellowfin goatfish	(Diamant, Colorni & Ucko 2004)
	Mullus surmuletus	Surmullet	(Padrós et al. 2001)
Nemipteridae	*Scolopsis ghanam*	Arabian moocle bream	(Diamant, Colorni & Ucko 2004)
Oplegnathidae	*Oplegnathus punctatus*	Spotted knifejaw	(Yanagida et al. 2008)
Paralichthyidae	*Paralichthys olivaceus*	Olve flounder	(Sekiya et al. 2016; Yasuda et al. 2005)
Pleuronectidae	*Platichthys stellatus*	Starry flounder	(Shin & Lee 2023)
Pomacentridae	*Amphiprion clarkia*	Anemonefish	(Yokoyama & Shirakashi 2007)
	Amphiprion ocellaris	Clownfish anemonefish	(Yokoyama & Shirakashi 2007)
	Chromis chromis	Damselfish	(Özer et al. 2014; Padrós et al. 2001)
	Neopomacentrus miryae	Miry's demoiselle	(Diamant, Colorni & Ucko 2004)
Pomacanthidae	*Pomacanthus imperator*	Emperor angelfish	(Hyatt et al. 2018)

Family	Species	Common name	Reference
Scaridae	*Sparisoma cretense*	Parrotfish	(Katharios et al. 2014)
Sciaenidae	*Sciaenops ocellatus*	Red drum	(Diamant 1998b)
Scophthalmidae	*Scophthalmus maximus*	Turbot	(Sekiya et al. 2016)
Scorpaenidae	*Scorpaena porcus*	Black scorpionfish	(Padrós et al. 2001)
Sparidae	*Diplodus noct*	Red Sea seabream	(Diamant, Colorni & Ucko 2004)
	Diplodus puntazzo	Sharpsnout seabream	((Diamant 1995) cited in (Athanassopoulou, Prapas & Rodger 1999; Diamant 1997; Rigos et al.)
	Diplodus sargus	White seabream	(Padrós et al. 2001)
	Diplodus vulgaris	Common two-banded seabream	(Padrós et al. 2001)
	Pagellus erythrinus	Common pandora	(Alvarez-Pellitero 2004)
	Pagrus major	Common sea bream	((Diamant 1995); cited in Diamant 1997 #5943})(Le Breton & Marques 1995)
	Pagrus pagrus	Red porgy	(Alvarez-Pellitero 2004)
	Sparus aurata	Gilthead sea bream	(Diamant 1992; Sakiti et al. 1996)
	Spicara maena	Blotched picarel	(Padrós et al. 2001)
Tetraodontidae	*Takifugu niphoblesc*	Grass puffer	(Yokoyama & Shirakashi 2007)
	Takifugu rubripes	Tiger puffer	(Tun et al. 2000; Yasuda et al. 2002)
Cichlidae	*Astronotus ocellatus*	Oscar	(Diamant, Ram & Paperna 2006)
	Oreochromis mossambicus	Mozambique tilapia	(Diamant, Ram & Paperna 2006)
Cyprinidae	*Puntius tetrazona*	Tiger barb	(Diamant, Ram & Paperna 2006)
Danionidae	*Danio rerio*	Zebra fish	(Diamant, Ram & Paperna 2006)

Megalocytivirus

The World Organisation for Animal Health (WOAH) ad hoc Group on Susceptibility of fish species to infection with WOAH listed diseases (the ad hoc Group) undertook assessments in accordance with criteria described in Chapter 1.5 of the WOAH Aquatic animal health code (WOAH Code) (WOAH 2023a) for infection with red sea bream iridovirus (RSIV) (WOAH 2023d). Given that RSIV, infectious spleen and kidney necrosis virus (ISKNV) and turbot reddish body iridovirus (TRBIV) are closely related genogroups in the Genus *Megalocytivirus*, and that there is likely to be an overlap with RSIV in its epidemiology, pathology and diagnostic test methods, the ad hoc Group undertook assessments of susceptible species to *Megalocytivirus* (excluding scale drop disease virus (SDDV)).

In accordance with the *Report of the WOAH ad hoc Group on susceptibility of fish species to infection with WOAH listed diseases* (WOAH 2022c), the species showed in Table 27 meet the criteria for listing as susceptible to infection with *Megalocytivirus* (excluding SDDV) (WOAH 2023a). These species were proposed by the WOAH ad hoc group to be included in article 10.8.2 of a revised chapter 10.8 Infection with *Megalocytivirus* (WOAH 2023d). For details of the assessment please refer to the report of the ad hoc Group found in the WOAH webpage (WOAH 2022c).

Table 27 Species that meet the criteria for listing as susceptible to infection with *Megalocytivirus* (excluding SDDV)

Family	Scientific name	Common name	Assessed for
Apogonidae	*Pterapogon kauderni*	Banggai cardinalfish	ISKNV (genogroup)
Butidae	*Oxyeleotris marmorata*	marble goby	RSIV (genogroup)
			ISKNV (genogroup)
Carangidae	*Pseudocaranx dentex*	white trevally	Megalocytivirus (excluding SDDV)
	Seriola dumerili	greater amberjack	Megalocytivirus (excluding SDDV)
	Seriola lalandi	goldstripe amberjack, yellowtail kingfish	Megalocytivirus (excluding SDDV)
	Seriola quinqueradiata	Japanese amberjack	RSIV (genogroup)
	Seriola quinqueradiata x Seriola lalandi	Buri-hira hybrid	Megalocytivirus (excluding SDDV)
	Trachinotus blochii	snubnose pompano	Megalocytivirus (excluding SDDV)
	Trachinotus carolinus	Florida pompano	RSIV (genogroup)
	Trachurus japonicus	Japanese jack mackerel	Megalocytivirus (excluding SDDV)
Centrarchidae	*Lepomis macrochirus*	bluegill	RSIV (genogroup)
Cichlidae	*Astronotus ocellatus*	Oscar	RSIV (genogroup)
			ISKNV (genogroup)
	Etroplus suratensis	pearlspot	ISKNV (genogroup)
	Oreochromis niloticus	Nile tilapia	RSIV (genogroup)
	Pterophyllum altum	deep angelfish	ISKNV (genogroup)
	Pterophyllum scalare	freshwater angelfish	ISKNV (genogroup)
Cyprinidae	*Epalzeorhynchos frenatum*	rainbow sharkminnow	ISKNV (genogroup)
Danionidae	*Danio rerio*	zebrafish	ISKNV (genogroup)
Ephippidae	*Platax orbicularis*	orbiculate batfish	ISKNV (genogroup)

Family	Scientific name	Common name	Assessed for
Girellidae	*Girella punctata*	largescale blackfish	Megalocytivirus (excluding SDDV)
Haemulidae	*Parapristipoma trilineatum*	chicken grunt	Megalocytivirus (excluding SDDV)
	Plectorhinchus cinctus	crescent sweetlips	Megalocytivirus (excluding SDDV)
Latidae	*Lates calcarifer*	barramundi	RSIV (genogroup)
			ISKNV (genogroup)
			RSIV (genogroup)
Lethrinidae	*Lethrinus haematopterus*	Chinese emperor	Megalocytivirus (excluding SDDV)
	Lethrinus nebulosus	spangled emperor	Megalocytivirus (excluding SDDV)
Mugilidae	*Mugil cephalus*	flathead grey mullet	Megalocytivirus (excluding SDDV)
Nothobranchiidae	*Aphyosemion gardneri*	steel blue killifish	ISKNV (genogroup)
Oplegnathidae	*Oplegnathus fasciatus*	barred knifejaw	RSIV (genogroup)
			ISKNV (genogroup)
	Oplegnathus punctatus	spotted knifejaw	RSIV (genogroup)
			ISKNV (genogroup)
Osphronemidae	*Macropodus opercularis*	paradise fish	RSIV (genogroup)
	Osphronemus goramy	giant gourami	ISKNV (genogroup)
	Trichogaster lalius	dwarf gourami	ISKNV (genogroup
			TRBIV (genogroup)
	Trichopodus leerii	pearl gourami	ISKNV (genogroup)
	Trichopodus microlepis	moonlight gourami	ISKNV (genogroup)
Paralichthyidae	*Paralichthys olivaceus*	bastard halibut	RSIV (genogroup)
			TRBIV (genogroup)
			Megalocytivirus (excluding SDDV)
Percichthyidae	*Maccullochella peelii*	Murray cod	ISKNV (genogroup)
Pleuronectidae	*Verasper variegatus*	spotted halibut	Megalocytivirus (excluding SDDV)
Poeciliidae	*Poecilia latipinna*	sailfin molly	ISKNV (genogroup)
	Poecilia reticulata	guppy	Megalocytivirus (excluding SDDV)
	Xiphophorus hellerii	green swordtail	ISKNV (genogroup)
	Xiphophorus maculatus	southern platyfish	ISKNV (genogroup)
Procatopodidae	*Poropanchax normani*	Norman's lampeye	Megalocytivirus (excluding SDDV)
Rachycentridae	*Rachycentron canadum*	Cobia	Megalocytivirus (excluding SDDV)
Sciaenidae	*Larimichthys crocea*	large yellow croaker	RSIV (genogroup)
	Sciaenops ocellatus	red drum	ISKNV (genogroup)
Scombridae	*Scomber japonicus*	chub mackerel	Megalocytivirus (excluding SDDV)
	Scomberomorus niphonius	Japanese Spanish mackerel	Megalocytivirus (excluding SDDV)
	Thunnus orientalis	Pacific bluefin tuna	Megalocytivirus (excluding SDDV)
Scophthalmidae	*Scophthalmus maximus*	turbot	TRBIV (genogroup)
Serranidae	*Epinephelus akaara*	Hong Kong grouper	Megalocytivirus (excluding SDDV)
	Epinephelus awoara	yellow grouper	Megalocytivirus (excluding SDDV)

Family	Scientific name	Common name	Assessed for
	Epinephelus bruneus	longtooth grouper	Megalocytivirus (excluding SDDV)
	Epinephelus coioides	orange-spotted grouper	Megalocytivirus (excluding SDDV)
	Epinephelus fuscoguttatus	brown-marbled grouper	Megalocytivirus (excluding SDDV)
	Epinephelus fuscoguttatus ♀× ♂ *E. lanceolatus*	pearl gentian grouper (hybrids)	ISKNV (genogroup)
	Epinephelus malabaricus	Malabar grouper	Megalocytivirus (excluding SDDV)
	Epinephelus septemfasciatus	convict grouper	Megalocytivirus (excluding SDDV)
Sinipercidae	*Siniperca chuatsi*	Mandarin fish	RSIV (genogroup)
			ISKNV (genogroup)
Sparidae	*Acanthopagrus schlegelii*	blackhead seabream	RSIV (genogroup)
	Dentex tumifrons	yellowback seabream	Megalocytivirus (excluding SDDV)
	Pagrus major	red sea bream	RSIV (genogroup)
Stromateidae	*Pampus argenteus*	silver pomfret	RSIV (genogroup)
Synanceiidae	*Inimicus japonicus*	no common name	RSIV (genogroup)
Tetraodontidae	*Takifugu rubripes*	tiger pufferfish	Megalocytivirus (excluding SDDV)

Source: Adapted from (WOAH 2022c)

Species assessed by the WOAH ad hoc group as having incomplete evidence of susceptibility to infection with *Megalocytivirus* in accordance with chapter 1.5 of the WOAH Code, are listed in Table 28 (WOAH 2022c). The ad hoc Group has proposed these species to be included in section 2.2.2 of a revised chapter 2.3.7 *Infection with Megalocytivirus* of the WOAH Manual of diagnostic tests for aquatic animals (WOAH Manual).

Table 28 Species with incomplete evidence of susceptibility to infection with *Megalocytivirus* (excluding SDDV)

Family	Scientific name	Common name	Assessed for
Cichlidae	*Cleithracara maronii*	keyhold cichlid	TRBIV (genogroup)
	Mikrogeophagus ramirezi	ram cichlid	ISKNV (genogroup)
	Pterophyllum scalare	freshwater angelfish	TRBIV (genogroup)
Helostomatidae	*Helostoma temminckii*	kissing gourami	ISKNV (genogroup)
Lateolabracidae	*Lateolabrax japonicus*	Japanese seabass	RSIV (genogroup)
Oplegnathidae	*Oplegnathus fasciatus*	barred knifejaw	TRBIV (genogroup)
Osphronemidae	*Betta splendens*	siamese fighting fish	Megalocytivirus (excluding SDDV)
	Trichopodus leerii	pearl gourami	RSIV (genogroup)
	Trichopodus trichopterus	three spot gourami	ISKNV (genogroup)
			TRBIV (genogroup)
Poeciliidae	*Poecilia sphenops*	molly	ISKNV (genogroup)
	Poecilia velifera	sail-fin molly	Megalocytivirus (excluding SDDV)
	Xiphophorus variatus	variable platyfish	ISKNV (genogroup)
Sebastidae	*Sebastes schlegeli*	rockfish	RSIV (genogroup)

Family	Scientific name	Common name	Assessed for
Sparidae	*Rhabdosargus sarba*	goldlined seabream	RSIV (genogroup)
			ISKNV (genogroup)

Source: Adapted from (WOAH 2022c).

In accordance with the WOAH ad hoc group, the species in which pathogen-specific PCR results for infection with *Megalocytivirus* had been reported, but an active infection had not been demonstrated, are listed in Table 29. These species were proposed by the WOAH ad hoc Group to be included in section 2.2.2 of a revised chapter 2.3.7 *Infection with Megalocytivirus* in the WOAH Manual.

Table 29 Species in which pathogen-specific PCR results for infection with *Megalocytivirus* (excluding SDDV) had been reported, but an active infection had not been demonstrated

Family	Scientific name	Common name	Assessed for
Carangidae	*Alepes djedaba*	shrimp scad	ISKNV (genogroup)
	Caranx sexfasciatus	bigeye trevally	ISKNV (genogroup)
	Decapterus maruadsi	Japanese scad	ISKNV (genogroup)
	Scomberoides lysan	doublespotted queenfish	ISKNV (genogroup)
	Scomberoides tala	barred queenfish	ISKNV (genogroup)
	Selaroides leptolepis	yellowstripe scad	ISKNV (genogroup)
Characidae	*Moenkhausia costae*	tetra fortune	Megalocytivirus (excluding SDDV)
Clupeidae	*Konosirus punctatus*	dotted gizzard shad	ISKNV (genogroup)
Cobitidae	*Misgurnus anguillicaudatus*	pond loach	Megalocytivirus (excluding SDDV)
Cynoglossidae	*Cynoglossus sinicus*	no common name	ISKNV (genogroup)
Cyprinidae	*Carassius auratus*	goldfish	ISKNV (genogroup)
	Cyprinus carpio	common carp	ISKNV (genogroup)
Danionidae	*Danio albolineatus*	pearl danio	ISKNV (genogroup)
Engraulidae	*Thryssa mystax*	moustached thryssa	ISKNV (genogroup)
Haemuliae	*Plectorhinchus pictus*	trout sweetlips	ISKNV (genogroup)
Hemiodontidae	*Hemiodus gracilis*	no common name	Megalocytivirus (excluding SDDV)
Leiognathidae	*Deveximentum insidiator*	pugnose ponyfish	ISKNV (genogroup)
	Leiognathus brevirostris	shortnose ponyfish	ISKNV (genogroup)
	Leiognathus equulus	common ponyfish	RSIV (genogroup)
	Photopectoralis bindus	orangefin ponyfish	ISKNV (genogroup)
Loricariidae	*Hypostomus plecostomus*	suckermouth catfish	Megalocytivirus (excluding SDDV)
Lutjanidae	*Lutjanus argentimaculatus*	mangrove red snapper	ISKNV (genogroup)
	Lutjanus johnii	John's snapper	ISKNV (genogroup)
	Lutjanus russelli	Russell's snapper	ISKNV (genogroup)
	Lutjanus sanguineus	humphead snapper	ISKNV (genogroup)
Monacanthidae	*Paramonacanthus japonicus*	hair-finned leatherjacket	ISKNV (genogroup)
Osphronemidae	*Macropodus opercularis*	paradise fish	ISKNV (genogroup)

Family	Scientific name	Common name	Assessed for
	Trichogaster labiosa	thick lipped gourami	Megalocytivirus (excluding SDDV)
Osteoglossidae	*Arapaima gigas*	araipama	Megalocytivirus (excluding SDDV)
Pangasiidae	*Pangasianodon hypothalymus*	striped catfish	Megalocytivirus (excluding SDDV)
Polynemidae	*Eleutheronema tetradactylum*	fourfinger threadfin	ISKNV (genogroup)
Pomacanthidae	*Pomacanthus navarchus*	bluegirdled anglefish	Megalocytivirus (excluding SDDV)
Sciaenidae	*Dendrophysa russelii*	goatee croaker	ISKNV (genogroup)
	Otolithes ruber	tigertooth croaker	ISKNV (genogroup)
	Pennahia argentata	silver croaker	ISKNV (genogroup)
	Pennahia macrocephalus	big-head pennah croaker	ISKNV (genogroup)
Scombridae	*Scomberomorus commerson*	narrow-barred Spanish mackerel	ISKNV (genogroup)
Serranidae	*Cephalopholis boenak*	chocolate hind	ISKNV (genogroup)
	Epinephelus bleekeri	duskytail grouper	ISKNV (genogroup)
	Epinephelus chlorostigma	brownspotted grouper	ISKNV (genogroup)
	Epinephelus fasciatomaculosus	rock grouper	ISKNV (genogroup)
	Epinephelus lanceolatus	giant grouper	RSIV (genogroup)
	Epinephelus merra	honeycomb grouper	ISKNV (genogroup)
Serrasalmidae	*Pygocentrus nattereri*	red piranha	ISKNV (genogroup)
	Serrasalmus gibbus	no common name	Megalocytivirus (excluding SDDV)
Siganidae	*Siganus canaliculatus*	rabbitfish	ISKNV (genogroup)
Stromateidae	*Pampus argenteus*	silver pomfret	ISKNV (genogroup)
Synodontidae	*Saurida elongata*	slender lizardfish	ISKNV (genogroup)
Syphyraenidae	*Sphyraena forsteri*	bigeye barracuda	ISKNV (genogroup)
Terapontidae	*Pelates quadrilineatus*	fourline grunter	ISKNV (genogroup)
	Terapon jarbua	Jarbua terapon	ISKNV (genogroup)
Tetraodontidae	*Lagocephalus spadiceus*	half-smooth golden pufferfish	ISKNV (genogroup)
	Takifugu alboplumbeus	no common name	ISKNV (genogroup)
	Takifugu xanthopterus	yellowfin pufferfish	ISKNV (genogroup)

Source: Adapted from (WOAH 2022c).

Other species in which pathogen-specific PCR results for infection with *Megalocytivirus* had been reported (and not listed in the report of the WOAH ad hoc Group), are listed in Table 30.

Table 30 Other species in which pathogen-specific PCR results for infection with *Megalocytivirus* had been reported

Family	Scientific name	Common name	Reference
Apogonidae	*Cheilodipterus quinquelineatus*	five-lined cardinalfish	(Becker et al. 2017)
	Ostorhinchus compressus	ochre-striped cardinalfish	
Pomacentridae	*Amphiprion sebae*	sebae anemonefish	

Family	Scientific name	Common name	Reference
	Amphiprion perideraion	pink anemonefish	(Becker et al. 2017; United States Department of Agriculture 2022)
	Amphiprion ocellaris	clown anemonefish	
	Chromis viridis	blue green damselfish	
Scorpaenidae	*Pterois volitans*	Lionfish	(United States Department of Agriculture 2022)

Nervous Necrosis Virus

Table 31 lists species which are susceptible to infection with BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV.

Table 31 Species susceptible to BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV

Family	Scientific name	Common name	Genotype	
Carangidae	*Pseudocaranx dentex*	white trevally	SJNNV	(Mori et al. 1992)
	Trachurus japonicus	Japanese jack mackerel	SJNNV	(Nishioka et al. 2016)
Gadidae	*Gadus macrocephalus*	Pacific cod	BFNNV	(Mao et al. 2015)
	Gadus morhua	Atlantic cod	BFNNV	(Johnson et al. 2002; Nylund et al. 2008)
	Melanogrammus aeglefinus	haddock	BFNNV	(Gagne et al. 2004)
Moronidae	*Dicentrarchus labrax*	European seabass	BFNNV, SJNNV, RGNNV/SJNNV	(Athanassopoulou et al. 2003; Cutrín et al. 2007; Thiery, Cozien & de Boisseson 2004)
Paralichthyidae	*Paralichthys olivaceus*	bastard halibut	BFNNV, TPNNV	(Iwamoto et al. 2000; Nishizawa et al. 1997)
Pleuronectidae	*Hippoglossus hippoglossus*	Atlantic halibut	BFNNV	(Grotmol et al. 1997; Starkey et al. 2000)
	Pseudopleuronectes americanus	winter flounder	BFNNV	(Barker et al. 2002)
	Verasper moseri	Barfin flounder	BFNNV	(Muroga 1995).
Salmonidae	*Salmo salar*	Atlantic salmon	BFNNV	(Korsnes et al. 2005)
Scophthalmidae	*Scophthalmus maximus*	turbot	BFNNV	(Bloch, Gravningen & Larsen 1991)
Soleidae	*Solea senegalensis*	Senegalese sole	SJNNV RGNNV/SJNNV	(Cutrín et al. 2007; Olveira et al. 2009; Thiery, Cozien & de Boisseson 2004)
	Solea solea	common sole	BFNNV	(Starkey et al. 2001)
Sparidae	*Sparus aurata*	gilthead bream	SJNNV/RGNNV, RGNNV/SJNNV	(Cutrín et al. 2007; Olveira et al. 2009; Toffan et al. 2017; Volpe et al. 2020)
Tetraodontidae	*Takifugu rubripes*	tiger pufferfish	TPNNV	(Cutrín et al. 2007; Olveira et al. 2009; Toffan et al. 2017; Volpe et al. 2020)

BFNNV Barfin flounder nervous necrosis virus; **NNV** nervous necrosis virus genotype not determined; **SJNNV** striped jack nervous necrosis virus; **RGNNV** red-spotted grouper nervous necrosis virus; **TPNNV** tiger puffer nervous necrosis virus

Table 32 lists species for which RT-PCR-positive results have been reported for BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV but no active infection has been demonstrated.

Table 32 Species for which RT-PCR-positive results have been reported for BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV but no active infection has been demonstrated

Family	Scientific name	Common name	Genotype	Reference
Acanthuridae	*Zebrasoma flavescens*	yellow tang	NNV	(Gomez et al. 2006)
Anguillidae	*Anguilla anguilla*	European eel	SJNNV	(Bandín et al. 2014)
Carangidae	*Carangoides equula*	whitefin trevally	SJNNV	(Sakamoto et al. 2008)
	Selene vomer	lookdown	NNV	(Gomez et al. 2006)
	Seriola dumerili	greater amberjack	SJNNV	(Sakamoto et al. 2008)
	Seriola quinqueradiata	Japanese amberjack	SJNNV	(Sakamoto et al. 2008)
	Trachurus mediterraneus	Mediterranean horse mackerel	RGNNV/SJNNV	(Bitchava et al. 2019)
Centriscidae	*Aeoliscus strig*atus	razorfish	NNV	(Gomez et al. 2006)
Chanidae	*Chanos chanos*	milkfish	NNV	(Gomez et al. 2006)
Dussumieriidae	*Etrumeus teres*	red-eye round herring	BFNNV	(Sakamoto et al. 2008)
Labridae	*Ctenolabrus rupestris*	goldsinny wrasse	BFNNV	(Korsnes et al. 2017)
	Labrus bergylta	ballan wrasse	BFNNV	(Korsnes et al. 2017)
	Symphodus melops	corking wrasse	BFNNV	(Korsnes et al. 2017)
Monocentridae	*Monocentris japonica*	pinecone fish	NNV	(Gomez et al. 2006)
Muraenidae	*Rhinomuraena quaesita*	ribbon moray	NNV	(Gomez et al. 2006)
Pomacentridae	*Dascyllus trimaculatus*	threespot dascyllus	NNV	(Gomez et al. 2006)
Sciaenidae	*Argyrosomus regius*	meagre	SJNNV	(Lopez-Jimena et al. 2010)
Scombridae	*Scomber japonicus*	chub mackerel	SJNNV	(Sakamoto et al. 2008)
Soleidae	*Solea solea*	common sole	RGNNV/SJNNV	(Panzarin et al. 2012)
Sparidae	*Evynnis cardinalis*	threadfin porgy	SJNNV	(Ma et al. 2015)
	Pagrus major	red sea bream	SJNNV	(Sakamoto et al. 2008)

BFNNV Barfin flounder nervous necrosis virus; **NNV** nervous necrosis virus genotype not determined; **SJNNV** striped jack nervous necrosis virus; **RGNNV** red-spotted grouper nervous necrosis virus; **TPNNV** tiger puffer nervous necrosis virus

Salmonid alphavirus

Species which fulfil the criteria for listing as a species susceptible to infection with SAV in accordance with chapter 1.5 of the WOAH Aquatic animal health code (WOAH Code) (WOAH 2023e) include those listed in Table 33.

Table 33 Species which fulfil the criteria for listing as a species susceptible to infection with SAV

Family	Scientific name	Common name	Reference
Pleuronectidae	*Limanda limanda*	Common dab flatfish	(Snow et al. ; Wallace, McKay & Murray 2017)
Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	(Fringuelli et al. 2008)
	Salmo salar	Atlantic salmon	(Fringuelli et al. 2008; Graham et al. 2010; Jansen et al. 2010a; McLoughlin, Rowley & Doherty 1998)
	Salvelinus alpinus	Arctic char	(Lewisch et al. 2018; Wallace, McKay & Murray 2017)

Species for which there is incomplete evidence for susceptibility to infection with SAV in accordance with chapter 1.5 of the WOAH Code (WOAH 2023e) are listed in Table 34.

Table 34 Species for which there is incomplete evidence for susceptibility to infection with SAV

Family	Scientific name	Common name	Reference
Labridae	*Labrus bergylta*	Ballan wrasse	(Ruane et al.)
Pleueonectidae	*Hippoglossoides platessoides*	Long rough dab	(Snow et al. ; Wallace, McKay & Murray 2017)
	Pleuronectes platessa	Plaice	(Snow et al. ; Wallace, McKay & Murray 2017)

SAV-positive PCR results have also been reported in several species; however no active infection was demonstrated are listed in Table 35.

Table 35 Species for which PCR-positive results have been reported for SAV but no active infection has been demonstrated

Family	Scientific name	Common name	Reference
Cottidae	*Myoxocephalus octodecemspinosus*	Longhorn sculpin	(WOAH)
Gadidae	*Melanogrammus aeglefinus*	Haddock	(Wallace, McKay & Murray 2017)
	Trisopterus esmarkii	Norway pout	(Wallace, McKay & Murray 2017)
	Pollachius virens	Saithe	(Wallace, McKay & Murray 2017)
	Merlangius merlangus	Whiting	(Wallace, McKay & Murray 2017)
	Gadus morhua	Atlantic cod	(Wallace, McKay & Murray 2017)
Merlucciidae	*Merlucciu hubbsi*	Argentine hake	(WOAH)
Clupeidae	*Clupea harengus*	Herring	(Wallace, McKay & Murray 2017)
Pleuronectidae	*Platichthys flesus*	European flounder	(Wallace, McKay & Murray 2017)
Salmonidae	*Salmo trutta*	Brown trout	(Wallace, McKay & Murray 2017)

Family	Scientific name	Common name	Reference
Caligidae	*Lepeophtheirus salmonis*	Sea lice copepod	(Petterson, Sandberg & Santi 2009)

Similar damselfish virus, and like ranaviruses

Species which are susceptible to infection with similar damselfish virus, and like ranaviruses, include but are not limited to, those listed in Table 36.

Table 36 Species which are susceptible to infection with similar damselfish virus, and like ranaviruses

Family	Scientific name	Common name	Ranavirus strain	Reference
Pomacentridae	*Pomacentrus similis*	Similar damselfish	SRDV	(Sivasankar et al. 2017b)
Latidae	*Lates calcarifer*	Barramundi	SRDV, KIRV	(Sivasankar et al. 2017b)
Cichlidae	*Oreochromis niloticus*	Nile tilapia	KIRV	(Kaviarasu et al. 2022)
Cyprinidae	*Cyprinus carpio*	Common koi	SRDV, KIRV	(George et al. 2015)
	Carassius auratus	Goldfish	KIRV	(Kaviarasu et al. 2022)
	Necomis leptocephalus	Bluehead chub	LMBV	(Iwanowicz et al. 2013)
Centrarchidae	*Miropterus salmoides*	Largemouth bass	LMBV	(Grizzle et al. 2002)
	Micropterus dolomieu	Smallmouth bass	LMBV	(Iwanowicz et al. 2013)
	Micropterus punctulatus	Spotted bass	LMBV	(Iwanowicz et al. 2013)
	Micropterus notius	Suwannee bass	LMBV	(Iwanowicz et al. 2013)
	Lepomis macrochirus	Bluegill	LMBV	(Iwanowicz et al. 2013)
	Lepomis auratus	Redbreast sunfish	LMBV	(Iwanowicz et al. 2013)
	Ambloplites rupestris	Rock bass	LMBV	(Iwanowicz et al. 2013)
	Pomoxis nigromaculatus	Black crappie	LMBV	(Iwanowicz et al. 2013)
Moronidae	*Morone mississippiensis*	Yellow bass	LMBV	(Iwanowicz et al. 2013)
	Morone americana	White perch	LMBV	(Iwanowicz et al. 2013)
Esocidae	*Esox masquinongy*	Muskellunge	LMBV	(Iwanowicz et al. 2013)
Sciaentidae	*Apoldinotus grunniens*	Freshwater frum	LMBV	(Iwanowicz et al. 2013)
Castostomidae	*Minytrema melanops*	Spotted sucker	LMBV	(Iwanowicz et al. 2013)
Channidae	*Channa argus*	Northern snake	LMBV	(Iwanowicz et al. 2013)

Viral haemorrhagic septicaemia virus

Species which are susceptible to infection with viral haemorrhagic septicaemia virus (VHSV) include, but are not limited to, to those listed in Table 37.

Table 37 Species which are susceptible to infection with VHSV

Family	Scientific name	Common name	Reference
Alosidae	*Alosa immaculata*	Pontic shad	(Ogut & Altuntas 2014)
	Alosa pseudoharengus	Alewife	(Stepien & Niner 2020)
	Sardina pilchardus	Pilchard	(Ogut & Altuntas 2014)
	Sardinops sagard	South American pilchard, sardine	(Hedrick et al. 2003; Traxler, Kieser & Richard 1999)
Ammodytidae	*Ammodytes personatus*	Sand eel	(Watanabe et al. 2002)
Argentinidae	*Argentina sphyraena*	Lesser Argentine	(Mortensen et al. 1999)
Carangidae	*Seriola dumerili*	Greater amberjack	(WOAH 2023f)
Caranginae	*Trachurus meditarraneus*	Mediterranean horse mackerel	(Ogut & Altuntas 2014)
Cyclopteridae	*Cyclopterus lumpus*	Lumpfish	(Gudmundsdóttir et al. 2019)
Epinephelidae	*Epinephelus akaara*	Hong Kong grouper	(Isshiki, Nagano & Miyazaki 2003)
Gadidae	*Gadiculus argenteus*	Silvery pout	(Sandlund et al. 2014)
	Gadus aeglefinus	Haddock	(Smail)
	Gadus chalcogrammus	Alaska pollock	(Meyers, Short & Lipson 1999)
	Gadus macrocephalus	Pacific cod	(Meyers et al. 1992)
	Gadus morhua	Atlantic cod	(Jensen et al. 1985)
	Melanogrammus aeglefinus	Haddock	(Smail 2000)
	Merlangius merlangus	Whiting	(Mortensen et al. 1999)
	Micromesistius poutassou	Blue whiting	(Mortensen et al. 1999)
	Trisoperus esmarkii	Norway pout	(Mortensen et al. 1999)
	Trisoperus minutus	Poor cod	(King et al. 2001)
Gaidropsaridae	*Enchelyopus cimbrius*	Fourbeard rockling	(Mortensen et al. 1999)
	Gaidropsarus vulgaris	Three-bearded rockling	(Ogut & Altuntas 2014)
Labridae	*Centrolabrus exoletus*	Rock cook wrasse	(Munro et al. 2015)
	Ctenolabrus rupestris	Goldsinny wrasse	(Munro et al. 2015)
	Labrus bergylta	Ballan wrasse	(Munro et al. 2015)
	Labrus mixtus	Cuckoo wrasse	(Munro et al. 2015)
	Symphodus melops	Corckwing wrasse	(Munro et al. 2015)
Liparidae	*Liparis tessellatus*	Cubed snailfish	(Lee et al. 2007)
Merlucciidae	*Merluccius productus*	Northern pacific hake	(Meyers, Short & Lipson 1999)
Mugilidae	*Mugil cephalus*	Flathead grey mullet	(Lee et al. 2007)
Mullidae	*Mullus barbatus*	Red mullet	(Ogut & Altuntas 2014)
Ophidiidae	*Hoplobrotula armata*	Armoured cusk	(Lee et al. 2007)

Family	Scientific name	Common name	Reference
Paralichthyidae	*Paralichthys olivaceus*	Bastard halibut	(Isshiki, Nagano & Suzuki 2001; Takano et al. 2001)
Pleuronectidae	*Glyptocephalus stelleri*	Blackfin flounder	(Lee et al. 2007)
	Hippoglossus hippoglossus	Atlantic halibut	(Bowden 2003)
	Limanda limanda	Common dab	(Skall, Olesen & Mellergaard 2005a)
	Pleuronectes platessus	European plaice	(Skall, Olesen & Mellergaard 2005b)
	Reinhardtius hippoglossoides	Greenland halibut	(Dopazo et al. 2002)
Rajidae	*Raja clavata*	Thornback ray	(Ogut & Altuntas 2014)
Sciaenidae	*Larimichthys polyactis*	Yellow croaker	(Lee et al. 2007)
Scombridae	*Scomber japonicus*	Pacific chub mackerel	(Hedrick et al. 2003)
Scorpaenidae	*Scorpaena izensis*	Izu scorpionfish	(Lee et al. 2007)
	Scorpaena porcus	Black scorpionfish	(Ogut & Altuntas 2014)
Scyliorhinidae	*Scyliorhinus torazame*	Claudy catshark	(Lee et al. 2007)
Soleidae	*Solea senegalensis*	Senegalese sole	(EFSA 2008)
Stromateidae	*Pampus argenteus*	Silver pomfret	(Lee et al. 2007)
Trichiuridae	*Trichiurus lepturus*	Largehead hairtail	(Lee et al. 2007)
Triglidae	*Eutrigla gurnardus*	Gray gurnard	(Wallace et al. 2015)
Uranoscopidae	*Uranoscopus scaber*	Atlantic stargazer	(Ogut & Altuntas 2014)
Ammodytidae	*Ammodytes hexapterus*	Pacific sand lance	(Kocan, Hershberger & Elder 2001)
Anguillidae	*Anguilla Anguilla*	European eel	((Castric et al. 1992) cited in (Skall, Olesen & Mellergaard 2005b))
Belonidae	*Belone belone*	Garfish	(Ogut & Altuntas 2014)
Clupeidae	*Clupea harengus*	Atlantic herring	(Dixon et al. 1997; Mortensen et al. 1999)
	Clupea harengus membras	Baltic herring	(Gadd et al. 2011)
	Clupea pallasii	Pacific herring	(Meyers et al. 1994)
	Sprattus sprattus	Sprat	(Mortensen et al. 1999)
Embiotocidae	*Cymatogaster aggregate*	Shiner perch	(Kent et al. 1998; Meyers & Winton 1995)
Engraulidae	*Engraulis encrasicolus*	European anchovy	(Ogut & Altuntas 2014)
Fundulidae	*Fundulus heteroclitus*	Mummichog	(Gagné et al. 2007)
Gasterosteidae	*Gasterosteus aculeatus*	Three spined stickleback	(Kent et al. 1998)
Gobiidae	*Neogobius melanostomus*	Round goby	(Groocock et al. 2007)
	Pomatoschistus minutus	Sand goby	(Skall, Olesen & Mellergaard 2005a)
Moronidae	*Dicentrarchus labrax*	Sea bass	(Castric & de Kinkelin 1984)
Osmeridae	*Hypomesus pretiosus*	Surf smelt	(Hedrick et al. 2003)

Family	Scientific name	Common name	Reference
	Thaleichthys pacificus	Eulachon	(Hedrick et al. 2003)
Pleuronectidae	*Platichthys flesus*	European flounder	(Skall, Olesen & Mellergaard 2005a)
Salmonidae	*Oncorhynchus kisutch*	Coho salmon	(Brunson, True & Yancey 1989)
	Oncorhynchus mykiss	Rainbow trout	(Castric & de Kinkelin 1980; Jensen 1965)
	Oncorhunchus mykiss x onchorhynchus kisutch hybrids	Rainbow trout x coho salmon hybrid	(Ord, Le Berre & de Kinkelin 1976)
	Oncorhynchus mykiss x Salvelinus alpinus hybrids	Rainbow trout x arctic char hybrids	(Dorson, Chevassus & Torhy 1991)
	Oncorhynchus mykiss x Salvelinus namaycush hybrids	Rainbow trout x lake trout hybrids	(Dorson, Chevassus & Torhy 1991)
	Oncorhynchus mykiss x Salmo trutta hybrids	Rainbow trout x brown trout hybrids	(WOAH 2023f)
	Oncorhynchus tshawytscha	Chinhook salmon	(Hopper 1989)
	Salmo salar	Atlantic salmon	(de Kinkelin & Castric 1982)
	Salmo truatta	Brown trout	(Jorgensen 1980)
Scophthalmidae	*Scophthalmus maximus*	Turbot	(Castric & de Kinkelin 1984; Schlotfeldt et al. 1991)
Sparidae	*Acanthopagrus schlegeli*	Blackhead seabream	(Isshiki, Nagano & Miyazaki 2003)
Adrianchthyidae	*Oryzias latipes*	Japanese rice fish	(Ito & Olesen 2013)
	Oryzias dancena	Marine medaka	(Kim, Oh & Oh 2013)
Catostomidae	*Castostomus commersonii*	White sucker	(WOAH 2023f)
Catostominae	*Moxostoma anisurum*	Silver redhorse	(Faisal et al. 2012)
	Moxostoma macrolepidotum	Shorthea readhorse	(Thompson et al. 2011)
Centrarchidae	*Ambloplites rupestris*	Rock bass	(Thompson et al. 2011)
	Lepomis gibbosus	Pumpkinseed	(Faisal & Winters 2011; Thompson et al. 2011)
	Lepomis macrochirus	Bluegill	(Faisal et al. 2012; USGS 2008)
	Micropterus dolomieu	Smallmouth bass	(Faisal et al. 2012; USGS 2008)
	Micropterus salmoides	Largemouth bass	((de Kinkelin et al. 1999) cited in (Skall, Olesen & Mellergaard 2005b))(Thompson et al. 2011)
	Pomoxis annuluris	White crappie	(Al-Hussinee et al. 2011)
	Pomoxis nigromaculatus	Black crappie	(Faisal et al. 2012; Thompson et al. 2011)
Cottidae	*Cottus pollux*	Japanese fluvial sculpin	(Ito & Olesen 2013)
Danionidae	*Danio rerio*	Zebra fish	(Novoa et al. 2006)
Dorosomatidae	*Dorosoma cepedianum*	American gizzard shard	(USGS 2008)

Family	Scientific name	Common name	Reference
Esocidae	*Esox Lucius*	Northern pike	((Meier & Jorgensen 1979) cited in (Skall, Olesen & Mellergaard 2005b))
	Esox Lucius x Esox masquinongy hybrids	Tiger muskellunge	(Getchell et al. 2013; Groocock et al. 2012)
	Esox masquinongy	Muskellunge	(Elsayed et al. 2006; Faisal et al. 2012)
Fundulidae	*Fundulus diaphanous*	Banded killifish	(Bain et al. 2010)
Gobiidae	*Rhinogobius sp.*	Yoshinobori	(Ito & Olesen 2013)
Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	(Thompson et al. 2011)
	Ictalurus punctuates	Channel catfish	(Thompson et al. 2011)
Leucisidae	*Notemigonus crysoleucas*	Golden shiner	(Cornwell & Bellmund 2013)
	Notropis atherinoides	Emerald shiner	(Faisal et al. 2012; USGS 2008)
	Notropis hudsonius	Spottail shiner	(Faisal et al. 2012)
	Pimephales notatus	Bluntnose minnow	(Frattini et al. 2011)
	Pimephales promelas	Fathead minnow	(Al-Hussinee et al. 2010)
	Semotilus corporalis	Fallfish	(WOAH 2023f)
Lotidae	*Lota lota*	Butbot	(Thompson et al. 2011)
Moronidae	*Morone americana*	White perch	(Thompson et al. 2011)
	Morone chrysops	White bass	(Thompson et al. 2011)
	Morone saxatilis	Striped bass	(Gagné et al. 2007)
Percidae	*Perca flavescens*	Yellow perch	(Kane-Sutton et al. 2010)
	Sander vitreus	Walleye	(Thompson et al. 2011)
Percopsidae	*Percopsis omiscomaycus*	Trout perch	(Thompson et al. 2011)
Petromyzontidae	*Lampetra fluviatilis*	River lamprey	(Gadd et al. 2010)
Salmonidae	*Coregonus artedii*	Lake cisco	(Thompson et al. 2011)
	Coreonus clupeaformis	Lake whitefish	(Thompson et al. 2011)
	Coregonus lavaretus	Common whitefish	(Skall et al. 2004)
	Coregonus sp.	Whitefish	(Ahne & Thomsen 1985)
	Oncorhynchus aguabonita	Golden trout	(Ahne, Negele & Ollenschlager 1976)
	Salmo marmoratus	Marble trout	(Pascoli et al. 2015)
	Salvelinus alpinus	Arctic char	(Dorson, Chevassus & Torhy 1991)
	Salvelinus fontinalis	Brook trout	(Ogut & Altuntas 2011; Rasmussen 1965)
	Salvelinus namaycush	Lake trout	(Dorson, Chevassus & Torhy 1991)
	Thymallys thymallyus	Grayling	((Wizigmann, Baath & Hoffmann 1980) cited in (Skall, Olesen & Mellergaard 2005b))
Sciaentidae	*Aplodinotus grunniens*	Freshwater drum	(Lumsden et al. 2007)

Species for which VHSV-positive RT-PCR results or viral isolation in cell culture have been reported, however, no active infection was demonstrated are listed in Table 38.

Table 38 Species for which VHSV-positive RT-PCR results or viral isolation in cell culture have been reported, however, no active infection was demonstrated

Family	Scientific name	Common name	Reference
Anoplopomatidae	*Anoplopoma fimbria*	Sabelfish	(Traxler, Kieser & Richard 1999)
Sparidae	*Sparus aurata*	Gilthead seabream	(Lopez-Vazquez et al. 2003)
Acipenseridae	*Acipenser baerii*	Siberian sturgeon	(Ryu et al. 2018)
Leuciscidae	*Pseudochondrostoma polylepis*	Iberian nace	(Lopez-Vazquez et al. 2003)

Appendix D Risk assessment values

Table 39 shows the risk assessment values for unrestricted import and (where applicable) restricted (with biosecurity measures applied) import for each hazard.

Table 39 Risk assessment values for each hazard

Hazard	Biosecurity measure	Likelihood of entry	Partial likelihood of exposure			Partial annual likelihood of entry and exposure			Partial likelihood of establishment and spread			Impact								Likely consequences			Partial annual risk			Annual risk
			Ornamental fish	Farmed foodfish	Wild fish	Ornamental fish	Farmed foodfish	Wild fish	Ornamental fish	Farmed foodfish	Wild fish	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect – social	Ornamental fish	Farmed foodfish	Wild fish	Ornamental fish	Farmed foodfish	Wild fish	All fish
A. sal	**Unrest**	**VL**	**EL**	**N**	**EL**	**EL**	**N**	**EL**	**EL**	**M**	**L**	**H**	**F**	**C**	**E**	**D**	**C**	**C**	**C**	**VL**	**H**	**M**	**N**	**N**	**N**	**N**
E. leei	**Unrest**	**H**	**M**	**EL**	**VL**	**M**	**EL**	**VL**	**L**	**VL**	**VL**	**VL**	**C**	**B**	**C**	**C**	**C**	**C**	**C**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
MCV	**Unrest**	M	H	EL	L	M	EL	L	M	**M**	M	M	E	D	E	E	C	E	D	M	M	M	M	N	L	M
	Free population	EL	H	EL	L	EL	N	EL	M	M	M	M	E	D	E	E	C	E	D	M	M	M	N	N	N	N
	Removal susceptible species	EL	H	EL	L	EL	N	EL	M	M	M	M	E	D	E	E	C	E	D	M	M	M	N	N	N	N
	Batch testing	VL	H	EL	L	VL	EL	VL	M	M	M	M	E	D	E	E	C	E	D	M	M	M	VL	N	VL	VL
NNV	**Unrest**	**VL**	**VL**	**EL**	**VL**	**EL**	**EL**	**EL**	**EL**	**M**	**L**	**H**	**F**	**C**	**E**	**D**	**D**	**A**	**D**	**VL**	**H**	**M**	**N**	**VL**	**N**	**VL**
SAV	**Unrest**	**VL**	**EL**	**EL**	**VL**	**EL**	**EL**	**EL**	**EL**	**M**	**L**	**H**	**F**	**C**	**E**	**D**	**D**	**A**	**C**	**VL**	**H**	**M**	**N**	**VL**	**N**	**VL**
SRDV	**Unrest**	**VL**	**VL**	**EL**	**VL**	**EL**	**EL**	**EL**	**EL**	**L**	**L**	**M**	**E**	**E**	**E**	**D**	**C**	**C**	**C**	**N**	**L**	**L**	**N**	**N**	**N**	**N**
VHSV	**Unrest**	**VL**	**EL**	**EL**	**VL**	**EL**	**EL**	**EL**	**EL**	**M**	**M**	**H**	**F**	**D**	**E**	**D**	**D**	**C**	**C**	**VL**	**H**	**H**	**N**	**VL**	**VL**	**VL**

Hazards: A. **sal** = *Aeromonas salmonicida* (typical strain); ***E. leei*** = *Enteromyxum leei*; **MCV** = *Megalocytivirus*; **SAV** = Salmonid alphavirus; **SRDV** = Similar damselfish ranavirus (SRDV) and closely related viral isolates (*i.e*. largemouth bass virus (LMBV) and koi ranavirus (KIRV)); **VHSV** = Viral haemorrhagic septicaemia virus; **NNV** = Nervous necrosis virus. **Biosecurity measure: Unrest** = Unrestricted risk, no biosecurity measures applied; **Free populations** = marine ornamental fish which have been sourced from a country, compartment or zone that is recognised by Australia to be free from *Megalocytivirus*; **Removal of susceptible species** = Removal of *Megalocytivirus* susceptible species from the permitted list; **Batch testing** = Pre-export batch testing for *Megalocytivirus*; **Risk rating: E** Extreme. **H** High. **M** Moderate. **L** Low. **VL** Very low. **EL** Extremely low. **N** Negligible. **Impact score** (**A, B, C, D, E, F**): See Figure 6.

Appendix E Additional health certification criteria and procedures for *Pterapogon* spp. and *Platax* spp. exported to Australia

All live *Pterapogon* spp. and *Platax* spp. fish present in the consignment must originate from a country, zone or compartment recognised by Australia to be free of megalocytiviruses (based on active surveillance), or batch tested prior to export under the supervision of an approved overseas competent authority and found to be negative for megalocytiviruses.

The additional health certification criteria and procedures for *Pterapogon* spp. and *Platax* spp. exported to Australia.

Sourced from a country, zone or compartment free of *Megalocytivirus*

The certification requirement that the fish originate from a country, zone or compartment determined by the Competent Authority to be free from *Megalocytivirus* must be based on active targeted surveillance in the country, zone or compartment.

Surveillance must demonstrate the absence of megalocytiviruses and suspected clinical signs in the source population during a surveillance period specified by the Competent Authority. The active targeted surveillance program must include a minimum of two rounds of sampling and testing for megalocytiviruses in the source population of fish to be exported to Australia. Chapter 1.4 *Aquatic animal health surveillance* of the World Organisation for Animal Health *Aquatic animal health code* (WOAH Code) should be referred to when designing a surveillance program for health certification purposes.

The Competent Authority may recognise a zone or compartment as free of megalocytiviruses based on negative test results using internationally recognised molecular diagnostic protocols (referred to in Diagnostic tests). The testing regimes apply in addition to the requirement for an absence of suspected clinical signs associated with megalocytiviruses, and that basic biosecurity conditions (defined in Basic biosecurity conditions) must have been continuously met during the surveillance period.

Sample size for source freedom

The sample size within a source population should be calculated using the guidelines in Chapter 1.4 *Aquatic animal health surveillance* of the WOAH Code. Source populations of fish to be included in a sampling regime may be of a single susceptible species if epidemiologically isolated, or if mixed populations of susceptible species, then these may be sampled using a stratified approach as described. Suggested sampling methodology is provided below for situations where disease prevalence in the source population is unknown or known.

Unknown prevalence

Should the health status of source fish be unknown then the sampling design of targeted active surveillance for demonstrating megalocytivirus-free status of a country, zone or compartment must require a minimum level of sampling appropriate for a 5% assumed prevalence with a 95% confidence level of detecting megalocytiviruses in a source population. Sample sizes for various combinations of design prevalence, diagnostic sensitivity and specificity values can be calculated online using Epitools Epidemiological Calculators.

Known prevalence

Should the Competent Authority be aware of the likely megalocytivirus prevalence in the source population (based on the previous baseline surveillance and/or published scientific literature), the sampling size can be designed with an expected prevalence that differs to 5%. The sampling design should consider epidemiological factors, including but not limited to, the virus characteristics (e.g. transmissibility, incubation period, the virus infecting homogenously or clustering within the population), population dynamics and biosecurity practices (e.g. the number of ponds/aquaria, physical structures of the farm, frequency and duration of population mixing, water intake and water sharing).

Diagnostic tests

The appropriate test methods for the purpose of demonstrating freedom of megalocytiviruses are those based on the polymerase chain reaction (PCR). These molecular tests must be capable of detecting subclinical carriers of the virus, such as two-step (nested) PCR and quantitative PCR methods published in peer-reviewed journals or equivalent. The standard PCR test for red seabream iridovirus (RSIV) described in the current WOAH *Manual of diagnostic tests for aquatic animals* (WOAH Manual) is not considered suitable at present as there is insufficient evidence that the test can detect the presence of megalocytiviruses in subclinically infected fish.

Ongoing surveillance

Ongoing surveillance to demonstrate disease freedom should involve twice yearly sampling and testing at a level appropriate for a 10% assumed prevalence with a 95% confidence level of detecting megalocytiviruses in a source population. The conditions for ongoing surveillance to demonstrate disease freedom will only be recognised following two years of continuous active targeted surveillance with no positive detections of megalocytiviruses, an absence of suspected clinical signs and demonstration that basic biosecurity conditions (defined in Basic biosecurity conditions) were continuously met for the period.

Basic biosecurity conditions

Basic biosecurity conditions are defined in the WOAH Code as a set of conditions applying to a particular disease, and a particular compartment, zone or country, required to ensure adequate disease security, such as:

- The disease, including suspicion of the disease, is compulsorily notifiable to the Competent Authority.
- An early detection system is in place within the compartment, zone or country.
- Import requirements to prevent the introduction of disease into the compartment, zone or country, as outlined in the WOAH Code, are in place.

Health certification

Exporting countries are required to provide health certification attesting to freedom from megalocytiviruses for all fish destined for export to Australia. This includes attestations of disease freedom for fish that may have been sourced from a different country to the final export country. Attestations of disease freedom in these circumstances must be based on satisfactory certification arrangements between the original source country and the final exporting country.

Pre-export batch testing

The certification requirement that the fish originate from a batch that has been tested by the Competent Authority and found negative for Megalocytivirus are based on those recommended for testing for *Megalocytivirus* for gouramis, bettas, paradise fish, cichlids and poeciliids and described in Pre-export batch testing.

These proposed requirements for testing of imported marine ornamental fish may be changed at any time if new information becomes available which suggests these measures do not manage risk to a level which achieves Australia's ALOP.

Pre-export batch testing for megalocytiviruses is not required if fish are sourced from a country, zone or compartment recognised by the Competent Authority of the exporting country to be free of the virus on the basis of a surveillance program consistent with Sourced from a country, zone or compartment free of Megalocytivirus.

Batch definition

For the purposes of batch testing for freedom from megalocytiviruses, a batch can be defined as fish of susceptible species sharing water in a single holding system at the time of sample collection that have remained epidemiologically isolated for at least 7 days from fish not of equivalent health status prior to export.

Diagnostic tests

Refer to the information in Diagnostic tests.

Sample size for batch testing

The sample size within a batch should be calculated using the guidelines in Chapter 1.4 *Aquatic animal health surveillance* of the WOAH Code. The design of a pre-export batch testing surveillance program for demonstrating freedom from megalocytiviruses must require a minimum level of sampling appropriate for a 5 % assumed prevalence. Sample sizes for various combinations of design prevalence, diagnostic sensitivity and specificity values can be calculated online using Epitools Epidemiological Calculators.

For some species, the number of animals constituting a batch is not large enough to meet the required sample size for pre-export batch testing. The Competent Authority of the exporting country may make an alternative arrangement for the suitable sample size for batch testing, either generally or case-by-case. Such arrangements should be supported by scientific data that would clearly demonstrate equivalence of the alternative arrangement.

All consignments of *Pterapogon* spp. and *Platax* spp. must be held in quarantine at the export premises while being sampled for testing and remain under biosecurity control at the export

premises until the results of the tests are available. Only fish from batches that return negative results are permitted to be exported to Australia.

Health certification for imported fish

Exporting countries are required to provide health certification attesting to freedom from megalocytiviruses for all fish destined for export to Australia. This includes attestations of disease freedom for fish that may have been sourced from a different country to the final export country. Attestations of disease freedom in these circumstances must be based on satisfactory certification arrangements between the original source country and the final exporting country.

Glossary

Term	Definition
appropriate level of protection (ALOP)	The Biosecurity Act 2015 defines the appropriate level of protection (or ALOP) for Australia as a high level of sanitary and phytosanitary protection aimed at reducing biosecurity risks to very low, but not to zero..
aquaculture	The farming of aquatic species under controlled conditions.
biosecurity	The prevention of the entry, establishment or spread of unwanted pests and infectious disease agents to protect human, animal or plant health or life, and the environment.
biosecurity measures	The Biosecurity Act 2015 defines biosecurity measures as measures to manage any of the following: biosecurity risk, the risk of contagion of a listed human disease, the risk of listed human diseases entering, emerging, establishing themselves or spreading in Australian territory, and biosecurity emergencies and human biosecurity emergencies.
biosecurity risk	The Biosecurity Act 2015 refers to biosecurity risk as the likelihood of a disease or pest entering, establishing or spreading in Australian territory, and the potential for the disease or pest causing harm to human, animal or plant health, the environment, economic or community activities.
captive bred fish	Fish that were spawned, hatched, settled and grown to juvenile or adult stage in an enclosed system.
coprophagy	The eating of faeces or excrement.
competent authority	The Veterinary Authority or other Government Authority of a Member Country having the responsibility and competence for ensuring or supervising the implementation of aquatic animal health and welfare measures, international health certification and other standards and recommendations in the OIE Aquatic Code in the whole territory.
endemic	Belonging to, native to, or prevalent in a particular geography, area or environment.
established	A self-sustaining pest or disease that occurs in Australia and is not regarded as eradicable. This definition builds on the Intergovernmental Agreement on Biosecurity definition of 'established'. An established pest or disease may be distributed widely across Australia or be only regionally distributed. A regionally distributed established pest or disease may be the subject of containment measures to mitigate further spread. Native or indigenous plants and animals, pests and diseases are not characterised as established for the purposes of this framework (even if having negative impacts, for example, overly abundant native fauna).
hazard	A biological, chemical or physical agent in, or a condition of, an aquatic animal or aquatic animal product with the potential to cause an adverse effect on aquatic animal health or public health.
hazard identification	Identification of potential hazards that may be associated with the importation of a commodity.
health certificate	For an animal or part of an animal that is to be imported into Australian territory from a place outside Australian territory (the overseas place) means a certificate that is in a form approved by the Director of Biosecurity and has been signed by an approved officer from the overseas place.
host	An organism that harbours a parasite, mutual partner or commensal partner, typically providing nourishment and shelter.
host range	Species capable, under natural conditions, of sustaining a specific pathogen or other organism.

import permit	Official document authorising a person to bring or import particular goods into Australian territory in accordance with specified import requirements.

mariculture	A branch of aquaculture involving the cultivation of marine species for food.
non-regulated risk analysis	Refers to the process for conducting a risk analysis that is not regulated under legislation (Biosecurity import risk analysis guidelines 2016).
ornamental fish	an aquatic animal that is intended for display, exhibition, competition, or to be kept as pet (WOAH 2023i).
pathway	Any means that allows the entry, exposure or spread of a hazard.
Pathogen	A biological agent that can cause disease to its host.
Aquatic pest	non-native aquatic plants or animals that harm, or have the potential to harm Australia's aquatic environment, social amenity or industries that use the aquatic environment, if they were to be introduced, established or spread. Aquatic pests include a wide range of salt and freshwater organisms from microscopic algae to species of seaweed, fish, barnacles, sea squirts, mussels and crabs.
quarantine	An establishment under the control of the Veterinary Authority where animals are maintained in isolation with no direct or indirect contact with other animals, to ensure that there is no transmission of specified pathogenic agents outside the establishment while the animals are undergoing observation for a specified length of time and, if appropriate, testing or treatment (OIE, 2022).
restricted risk	Risk estimate with phytosanitary measure(s) applied.
Risk analysis	Refers to the technical or scientific process for assessing the level of biosecurity risk associated with the goods, or the class of goods, and if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or class of goods to a level that achieves the ALOP for Australia.
risk assessment	The scientific evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a hazard.
risk management	The process of identifying, selecting and implementing measures that can be applied to reduce the level of risk.
stakeholders	Government agencies, individuals, community or industry groups or organisations, whether in Australia or overseas, including the proponent/applicant for a specific proposal, who have an interest in the policy issues.
surveillance	An official process that collects and records data on pest occurrence or absence by surveying, monitoring or other procedures (FAO 2017).
trophozoite	The active stage in the life cycle of an apicomplexan that feeds and multiplies.
unrestricted risk	Risks that apply in the absence of risk mitigation measures.

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