



Australian Government  
Department of Agriculture,  
Fisheries and Forestry

# Import of live sturgeon for aquaculture

## Draft biosecurity import risk analysis

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 (the department) has conducted this biosecurity import risk analysis (BIRA) to assess the biosecurity risks associated with the import of live *Acipenser* and *Huso* sturgeon and their reproductive material (eggs and milt) into Australia from all countries for aquaculture purposes.

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

Australia does not currently permit the import of any live sturgeon or their reproductive material. This BIRA proposes that live sturgeon and their reproductive material be permitted for import into Australia, subject to a range of biosecurity measures.

This BIRA identified hazards that require biosecurity measures to manage the risks to a level that achieves Australia's appropriate level of protection (ALOP). Table 1 lists the hazards and a summary of their relevant biosecurity measures.

**Table 1 Hazards and the biosecurity measures which when applied achieve Australia's appropriate level of protection**

Biosecurity measure achieve Australia's appropriate level of protection (yes/no/n/a)										
Hazard	Live							Reproductive material		
	Unrestricted (no biosecurity measures required)	Disease-free stock	PAQ with testing	Disease-free stock and PAQ with testing	Disease-free stock and PAQ parasite treatment	PEQ and PAQ parasite treatment	Unrestricted (no biosecurity measures required)	Disease-free stock	PAQ (with progeny production and testing)	Disease free stock and PAQ (with progeny production and testing)
NNV	yes	n/a	n/a	n/a	n/a	n/a	yes	n/a	n/a	n/a
<i>P. hydriforme</i>	no	yes	n/a	n/a	n/a	n/a	no	yes	n/a	n/a
AciHV1 and AciHV2	no	yes	yes	n/a	n/a	n/a	no	yes	yes	n/a
sNCLDV	no	yes	yes	n/a	n/a	n/a	no	yes	yes	n/a
CyHV-3 (KHV)	no	no	no	yes	n/a	n/a	no	no	no	yes
SVCV	no	no	no	yes	n/a	n/a	no	no	no	yes
IHN	no	no	no	yes	n/a	n/a	no	no	no	yes
VHSV	no	no	no	yes	n/a	n/a	no	no	no	yes
FV3	no	no	no	yes	n/a	n/a	no	no	no	yes
<i>A. salmonicida</i> (typical strain)	no	no	no	yes	n/a	n/a	no	no	no	yes
<i>Y. ruckeri</i> (Hagerman strain)	no	no	no	yes	n/a	n/a	no	no	no	yes
<i>Argulus</i> species	no	no	n/a	n/a	yes	yes	n/a	n/a	n/a	n/a
<i>E. sieboldi</i>	no	no	n/a	n/a	yes	yes	n/a	n/a	n/a	n/a



n/a not applicable because less restrictive or no biosecurity measures achieve Australia's appropriate level of protection.

**Hazard: AciHV1 and AciHV2** Acipenserid herpesvirus 1 and 2. **Argulus species** *Argulus alosae*, *Argulus coregoni*, *Argulus flavescentis*, *Argulus foliaceus* and *Argulus stizostheii*. **A. salmonicida (typical strain)** *Aeromonas salmonicida* (typical strain). **CyHV-3 (KHV)** Cyprinid herpesvirus 3 (koi herpesvirus). *E. sieboldi* *Ergasilus sieboldi*. **FV3** frog virus 3. **IHN** infectious haematopoietic necrosis virus. **P. hydriforme** *Polypodium hydriforme*. **sNCLDV** sturgeon nucleocytoplasmic large DNA viruses. **SVCV** spring viremia of carp virus. *Y. ruckeri* (**Hagerman strain**) *Yersinia ruckeri* (Hagerman strain). **VHSV** viral haemorrhagic septicaemia virus. **NNV** nervous necrosis virus. **Biosecurity measures: Unrestricted (no biosecurity measures required)** live sturgeon or their reproductive material with no biosecurity measures applied. **Disease-free stocks** live sturgeon or their reproductive material which have been sourced from a country, compartment or zone that is recognised by Australia to be free of the disease agent by active surveillance. **PAQ with testing** live sturgeon or sturgeon progeny are held under post-arrival quarantine with batch testing applied. **PEQ parasite treatment** live sturgeon are held under pre-export quarantine with parasite treatment applied. **PAQ parasite treatment** live sturgeon are held under post-arrival quarantine with parasite treatment applied. **PAQ (with progeny production and testing)** on-arrival sturgeon reproductive material is held under post-arrival quarantine for progeny production and resulting larvae/juveniles are batch tested.

When importing a new live species for aquaculture, the World Organisation for Animal Health typically recommend the first specimens imported (F0 generation) to remain indefinitely contained at a quarantine facility, with their subsequent generations being released. However, the slow development of sturgeon to reach sexual maturity (7 years+) does not make this feasible. Instead, alternative biosecurity measures are proposed to provide equivalent risk management to allow the safe release of the F0 generation.

The proposed biosecurity measures are conservative but considered necessary to protect the health of susceptible Australian fish and amphibians. This is because despite imported live sturgeon only being permitted to enter a recirculating aquaculture system under the *Environment Protection and Biodiversity Conservation Act 1999 Act*, the BIRA has assumed that the sturgeon may enter the Australian environment.

The department recognises that there might be new scientific information and technologies, or other combinations of measures that may provide an equivalent level of biosecurity protection for the hazards identified as requiring risk management in this draft report. Submissions supporting equivalence measures will be considered by the department on a case-by-case basis.

This draft report contains details of the risk assessment for each hazard and the proposed biosecurity measures to manage identified risks. Interested parties can provide comments and submissions to the department within the consultation period.

# Acronyms and abbreviations

**Table 2 Acronyms and abbreviations used in this biosecurity import risk analysis**

Term or abbreviation	Definition
AA	Approved Arrangement
ABARES	Australian Bureau of Agricultural and Resource Economics and Sciences
AciHV1	Acipenserid herpesvirus 1
AciHV2	Acipenserid herpesvirus 2
ALOP	Appropriate level of protection
BICON	Australia's Biosecurity Import Condition System
BIRA	Biosecurity import risk analysis
CA	Competent Authority
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FT	Flow-through systems
FV3	Frog virus 3
IUCN	International Union for Conservation of Nature
IHC	Immunohistochemistry
IHNV	Infectious haematopoietic necrosis virus
KHV	Koi herpesvirus
LSHV	Lake sturgeon herpesvirus
NACA	Network of Aquaculture Centres in Asia-Pacific
NNV	Nervous necrosis virus
WOAH	World Organisation for Animal Health
WOAH Code	WOAH Aquatic Animal Health Code
WOAH Manual	WOAH Manual of Diagnostic Tests and Vaccines for Aquatic Animals
PAQ	Post-arrival quarantine
PCR	Polymerase chain reaction
PEQ	Pre-export quarantine
PFU	Plaque-forming unit
RAS	Recirculating aquaculture systems
Real-time PCR	Real-time (or quantitative) polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse-transcription-polymerase chain reaction
SPS Agreement	World Trade Organization agreement on the Application of Sanitary and Phytosanitary Measures
sNCLDV	Sturgeon nucleocytoplasmic large DNA viruses

## Import of live sturgeon for aquaculture

Term or abbreviation	Definition
TCID <sub>50</sub>	50% tissue culture infective dose
VER	Viral encephalopathy and retinopathy
VHSV	Viral haemorrhagic septicaemia virus
WTO	World Trade Organization

# 1 Introduction

## 1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests and diseases entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, agricultural and aquaculture industries and human health.

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 (the department) 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. Successive Australian Governments have maintained a conservative approach to the management of biosecurity risks. The Appropriate Level of Protection (or ALOP) for Australia is defined as a high level of sanitary and phytosanitary protection aimed at reducing biosecurity risks to a very low level, but not to zero. If the biosecurity risks for imported goods do not achieve Australia's ALOP, biosecurity measures are proposed to reduce the risks to at least very low. If the risks cannot be reduced to an acceptable level, the goods will not be imported into Australia until suitable measures are identified.

Australia's risk analyses are undertaken by the department using technical and scientific experts in relevant fields and involve opportunities for consultation with stakeholders at various stages during the process.

Risk analyses may take the form of a biosecurity import risk analysis (BIRA) or a non-regulated risk analysis (such as scientific review of existing policy and import conditions, or scientific advice). The risk analysis on the import of live sturgeon or their reproductive material for aquaculture purposes was conducted as a BIRA.

Further information about Australia's biosecurity framework is provided in the [Biosecurity Import Risk Analysis Guidelines 2016](#).

### 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 animal, terrestrial animal, and 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 animal or animal product. Importantly, biosecurity measures are selected based on whether they reduce risk to a level that achieves Australia's ALOP. We do this in line with our international rights and obligations and supported by risk assessments.

## **1.2 The biosecurity import risk analysis framework**

A BIRA is a science-based assessment of the biosecurity risks associated with the import of a particular good, which is provided for under law. Under the [Biosecurity Act 2015](#), a BIRA must be conducted in accordance with the process prescribed in the [Biosecurity Regulation 2016](#) and takes into account the matters set out in the [BIRA Guidelines 2016](#).

The BIRA process will assess whether the import of a product with no biosecurity measures applied (the unrestricted risk) poses an unacceptable biosecurity risk, and if so, will recommend risk management measures to manage the risks to a level that achieves Australia's ALOP. If the biosecurity risk cannot be reduced to a level which achieves ALOP by the application of risk management measures, import will not be permitted. The biosecurity measures recommended are based on science, are the least trade restrictive to manage the risk, are practical and meet our rights and obligations under the World Trade Organization (WTO) *Agreement on the application of sanitary and phytosanitary measures* (the SPS agreement). The biosecurity measures form the basis of any required import conditions. The import of live sturgeon or their reproductive material cannot occur until suitable import conditions have been developed. These import conditions will be included on an import permit issued by the department under the *Biosecurity Act 2015*.

### **1.2.1 Regulated steps in the biosecurity import risk analysis process**

Under the [Biosecurity Regulation 2016](#), the steps that must be undertaken when conducting a BIRA include:

- 1) The Director of Biosecurity must appoint a scientific advisory group (SAG).
- 2) The Director of Biosecurity must publish a notice on the department's website stating:
  - a) That a BIRA is commencing.
  - b) The opportunities for consultation that will occur during the BIRA process.
- 3) The Director of Biosecurity must prepare an Issues Paper and publish it on the department's website. The Issues Paper will set out background information about the request, the commodity/goods and some of the main matters that will be considered during the analysis.
- 4) The Director of Biosecurity must:
  - a) Prepare a draft BIRA report.
  - b) Publish on the department's website the draft report and an invitation to the public to provide submissions about the assessment of the level of biosecurity risk associated with the relevant goods or class of goods including proposed biosecurity measures for the goods to achieve ALOP within a period specified in the invitation.
  - c) The consultation period must be at least 60 calendar days, including the day the invitation is published.

- d) If the Director of Biosecurity considers that the public may not have a reasonable opportunity to consider the draft BIRA report, the period for public submissions may be extended only once for a period of up to 60 calendar days.
- 5) The Director of Biosecurity must prepare a provisional BIRA report and publish it on the department's website.
- 6) Within 30 calendar days of the provisional BIRA report's publication, a person may make a request to the Inspector-General of Biosecurity to review the process used to conduct the BIRA.
- 7) If a person requests a review of the process for conducting the BIRA, and the Inspector-General is satisfied that a review can proceed, the Inspector-General must tell the Director of Biosecurity, in writing, about the request. The Inspector-General must then conduct a review of the process for conducting the BIRA.
- 8) If the Inspector-General conducts a review of the process for conducting the BIRA, the Director of Biosecurity must consider any recommendations in their report and must publish a final BIRA review report.
- 9) If the Inspector-General is not requested to conduct a review of the process for conducting the BIRA, the Director of Biosecurity must publish the provisional BIRA report as the final BIRA report as soon as is practical to do so.

The final BIRA report must be published within 30 months from the day the notice announcing the BIRA was published, unless specific circumstances apply.

The Director of Biosecurity may publish a notice on the department's website to stop the counting of time for a BIRA if:

- the Director of Biosecurity is waiting for requested further information, research or expert advice, or
- the Director of Biosecurity is waiting for examination by the SAG of a requested part of the BIRA process, or
- a biosecurity circumstance of national or international significance has occurred.

If the Inspector-General reviews the process for conducting the BIRA, the time taken for the review does not count towards the 30-month time frame.

The 30-month time frame also may not be met if a biosecurity circumstance of national or international significance occurs.

Publication of the final BIRA report represents the end of the process. The biosecurity measures recommended in the final report will be the basis of any import conditions in import permits issued by the department.

Step 1 of the BIRA process, the appointment of the SAG, was completed on 14 November 2019. A notice of intention announcing the commencement of the sturgeon BIRA and the issues paper were published on the department's website on 21 June 2022, completing step 2 and 3, respectively. The publication of this draft report represents the completion of step 4.

## 1.3 This biosecurity import risk analysis

### 1.3.1 Background

In 2015, the then Australian Government Department of the Environment and Energy, added *Acipenser baerii* (Siberian sturgeon) and *Huso huso* (beluga sturgeon) to the *List of specimens taken to be suitable for live import* (live import list) under the [Environment Protection and Biodiversity Conservation Act 1999](#) (EPBC Act). The amendment of the live import list to include the 2 species of sturgeon is available at the [Federal Register of Legislation](#). Crosses or hybrids of *A. baerii* and *H. huso*, irrespective of generational distance from the original mating or wild ancestor, are prohibited import unless the specific hybrid type is added to the live import list in its own right. Further information on the environmental assessment process to amend the live import list can be found at the Department of Climate Change, Energy, the Environment and Water (DCCEEW) [website](#).

Under the EPBC Act, importation of *A. baerii* and *H. huso* requires an import permit issued by DCCEEW and is only permitted for commercial aquaculture in a secure recirculating aquaculture system (RAS) to manage the risk of sturgeon establishing as a pest species in the wild.

Before these species of live sturgeon can be imported into Australia, the biosecurity risks must be assessed by the department through a BIRA to ensure the import achieves Australia's ALOP. In 2016, the department intended to commence the BIRA process, but this was delayed due to a diversion of resources to manage the response to an outbreak of white spot disease in prawns in Australia.

### 1.3.2 Scope

The scope of this BIRA is to consider the biosecurity risks associated with the unrestricted importation of live sturgeon or their reproductive material from all countries for aquaculture purposes. Reproductive material includes unfertilised eggs, milt and fertilised eggs. *A. baerii* and *H. huso* are the only 2 sturgeon species currently approved for import into Australia under the EPBC Act. However, this BIRA is generic in nature and will consider the import of all species of sturgeon within the genera *Acipenser* and *Huso*. Table 3 lists the species currently in the *Acipenser* and *Huso* genera. The term 'sturgeon' is used throughout this document rather than the full description of '*Acipenser* and *Huso* species'.

**Table 3 *Acipenser* and *Huso* species**

Species name	Common name(s)
<i>Acipenser baerii</i>	Siberian sturgeon
<i>Acipenser brevirostrum</i>	Shortnose sturgeon
<i>Acipenser dabryanus</i>	Yangtze sturgeon, Dabry's sturgeon
<i>Acipenser fulvescens</i>	Lake sturgeon, Rock sturgeon
<i>Acipenser gueldenstaedtii</i>	Danube sturgeon, Russian sturgeon, Diamond sturgeon
<i>Acipenser medirostris</i>	Green sturgeon
<i>Acipenser mikadoi</i>	Sakhalin sturgeon
<i>Acipenser naccarii</i>	Adriatic sturgeon
<i>Acipenser nudipectus</i>	Fringed barbel sturgeon, Thorn sturgeon, Bastard sturgeon, Ship sturgeon, Spiny sturgeon
<i>Acipenser oxyrinchus</i>	Atlantic sturgeon
<i>Acipenser persicus</i>	Persian sturgeon

Species name	Common name(s)
<i>Acipenser ruthenus</i>	Sterlet sturgeon
<i>Acipenser schrenckii</i>	Amur sturgeon, Japanese sturgeon
<i>Acipenser sinensis</i>	Chinese sturgeon
<i>Acipenser stellatus</i>	Starry sturgeon, Stellate sturgeon
<i>Acipenser sturio</i>	Common sturgeon, European sea sturgeon, Atlantic sturgeon, Baltic sturgeon
<i>Acipenser transmontanus</i>	White sturgeon
<i>Huso dauricus</i>	Kaluga
<i>Huso huso</i>	Beluga, Beluga sturgeon, Giant sturgeon

Source: Fishbase ([FAMILY Details for Acipenseridae - Sturgeons \(fishbase.se\)](https://www.fishbase.org/FAMILYDETAILS.aspx?familyid=322))

This BIRA will not restrict its scope to secure RAS. It will consider that the imported sturgeon are cultured in land-based semi-open aquaculture systems, as they represent the highest biosecurity risk. Semi-open systems include any land-based aquaculture system where water is exchanged between the farm and a natural waterway such as flow through raceways and earthen pond systems.

This BIRA will consider the scenario where imported sturgeon may be cultured with other fish species in the same aquaculture facility. This is because co-cultured or polycultured fish could then be translocated to other semi-open systems or the wild depending on the aquaculture license conditions, representing a biosecurity risk.

This BIRA does not consider the pest risk of sturgeon on the Australian environment or native species. A pest risk assessment was conducted for *A. baerii* and *H. huso* when they were considered for inclusion on the live import list. A pest risk assessment will be similarly conducted by DCCEEW for the inclusion of any other sturgeon species. For more information on these assessments, please contact DCCEEW at [exotic.species@dcceew.gov.au](mailto:exotic.species@dcceew.gov.au).

This BIRA will consider the impacts of the sturgeon disease agents identified as hazards on the native Australian environment. For example, it will consider whether the hazard can infect endangered and threatened native species, which may lead to losses of Australian biodiversity.

### 1.3.3 Existing policy

#### Import policy

Import policy does not currently exist for the importation of live sturgeon or their reproductive material. The import conditions for these commodities, once finalised, will be on the [Australian Biosecurity Import Conditions \(BICON\)](https://www.dcceew.gov.au/biosecurity/bicon) website.

#### 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 government authorities are responsible for animal health and environmental controls within their individual jurisdictions. Once animals and animal products have been cleared for import at the Australian border by department biosecurity officers, legislation relating to fisheries resource management or animal health may be used by state and territory government authorities to control intra and interstate movement of animals and their products.



The listing or classification of a species as ‘noxious’, ‘controlled’, ‘prohibited matter’ or ‘exotic’ allows a state or territory to apply regulations to control the use and potential spread of that animal. States and territories that currently have sturgeon listed (or classified) as ‘noxious’, ‘controlled’, ‘prohibited matter’ or ‘exotic’ under their respective legislation include:

- Western Australia (*Acipenser* are listed as ‘noxious’ under the *Fish Resources Management Act 1994*; *H. huso* is not listed on the Western Australia noxious fish list)
- Queensland (*Acipenser* and *H. huso* are listed as ‘prohibited matter—noxious’ under the *Biosecurity Act 2014*)
- New South Wales (*Acipenser* and *H. huso* are listed as ‘prohibited matter’ under the *Biosecurity Act 2015*)
- Tasmania (*Acipenser* and *H. huso* are declared as ‘controlled’ under the *Inland Fisheries Act 1995*)
- Northern Territory (*Acipenser* and *H. huso* are listed as ‘noxious’ under the *Fisheries Regulations 1992*)
- South Australia (*Acipenser* are classified as ‘noxious’ under the *Fisheries Management Act 2007*; the exception is *Acipenser baerii* which along with *H. huso* are classified as ‘exotic’)
- Victoria (*Acipenser* and *H. huso* are listed as ‘noxious’ under the *Fisheries Act 1995*).

The Australian Capital Territory has not listed *Acipenser* species or *H. huso* as ‘noxious’, ‘controlled’ or ‘exotic’ under their legislation. Western Australia and Victoria have provisions under their legislation for permits to be issued to allow listed noxious species to be kept in some circumstances. As *A. baerii* and *H. huso* are classified as ‘exotic’ (and ‘aquaculture fish’) in South Australia, they may be held by a person but cannot be released, deposited or permitted to escape into any waters without a permit.

All state and territories have legislative controls on aquaculture production. Aquaculture operations are required to be licensed and approval must be obtained from state and territory government authorities on various management practices including water and waste disposal methods and the control of fish escapes. Licensed aquaculture operators may have disease control programs and report significant disease events but it is not mandatory in all states and territories.

It is the importer and the licensed aquaculture operator’s responsibility to identify and ensure compliance with all state and territory requirements.

### **1.3.4 Previous consultation**

On 21 June 2022, a notice of intention and Animal Biosecurity Advice 2022-A03 were released announcing the department’s intent to conduct the sturgeon BIRA and the release of the issues paper. Stakeholders were invited to provide comment or information on the issues paper during a 60-day consultation period. The department received 5 submissions from stakeholders. Those submissions were carefully considered when conducting the risk analysis and preparing this draft BIRA report.

In addition, the draft BIRA report was peer-reviewed by leading independent experts in fish diseases and risk assessment, to ensure that the methods and assumptions were appropriate and that the data and information were the best available.

The Department of Health was consulted to ensure that public health considerations were included in the development of biosecurity policies. Consultation with the Australian Chief Veterinary Officer was also carried out before the release of the draft BIRA report.

On 11 July 2023, Animal Biosecurity Advice 2023-A09 announced the release of the draft BIRA report. Stakeholders are invited to provide comment on the draft BIRA report during a 60-day consultation period, which will close on 11 September 2023.

### **1.3.5 Next steps**

The department will carefully consider submissions received on this draft BIRA report and may consult informally with stakeholders. The department will then prepare a provisional BIRA report, taking into account stakeholder comments. The SAG will review the provisional BIRA report, as well as submissions to the draft BIRA report, to ensure that the department has considered stakeholder comments and any new information provided appropriately. The department will take any recommendations made by the SAG regarding the provisional BIRA report into account before publishing the report on its website. The department will also notify registered stakeholders and the WTO about the release of the provisional BIRA report.

The last step in the regulated process is to publish a final BIRA report on the department's website along with a notice advising stakeholders of the release. The biosecurity measures recommended in the final BIRA report will be the basis for the import conditions and any import permits issued by the department.

## 2 Sturgeon industry

### 2.1 Sturgeon

The Acipenseriformes (sturgeon and paddlefish) are native to the Northern Hemisphere – around half of these species are found in Europe, mostly in the Ponto-Caspian region, one third in North America, and the rest in East Asia and Siberia (Billard & Lecointre 2001). The family of sturgeon, *Acipenseridae*, includes four genera containing 25 species; 17 belong to the genus *Acipenser* (sturgeon), 2 to the genus *Huso* (giant sturgeon), 3 to the genus *Scaphirhynchus* (shovel-nosed sturgeon) and 3 to the genus *Pseudoscaphirhynchus* (Aral shovelnoses) (Froese & Pauly 2022). There are also numerous sturgeon hybrids, both natural and bred, such as *Huso huso* × *Acipenser ruthenus* (*Bester sturgeon*) and *Acipenser gueldenstaedtii* × *Acipenser baerii* (Mugetti et al. 2020b).

In natural populations, sturgeon reproduce in freshwater and then most species migrate to the sea, either living in brackish water (Caspian, Azov, Black and Baltic Seas) or in full seawater on the oceanic continental shelf, although some populations have remained entirely freshwater (Billard & Lecointre 2001). Sturgeon are one of the largest fish appearing in freshwater, capable of growing to over one tonne during their lifetimes (UNODC 2016). They have a long life cycle, with sexual maturity occurring later in life (5–30 years). Adult males and females do not spawn on an annual basis (Chebanov & Galich 2013).

### 2.2 Conservation of sturgeon

Globally there has been a long history of commercial sturgeon fisheries, primarily for caviar products but also as food fish. Due to over-exploitation of both natural and enhanced sturgeon stocks for caviar production, along with serious habitat deterioration and loss of natural spawning sites, there has been a drastic decline in natural populations (Bronzi, Rosenthal & Gessner 2011; Jahrl 2013; Ruban & Khodorevskaya 2011). Most sturgeon assessed for the *International Union for Conservation of Nature (IUCN) Red list of threatened species* are listed as endangered or critically endangered (IUCN 2020). *Acipenser brevirostrum*, *Acipenser oxyrinchus* and *Acipenser transmontanus* are listed as vulnerable and *Acipenser dabryanus* is listed as extinct in the wild (IUCN 2020).

In 1997, the IUCN listed all commercially utilised sturgeon species world-wide in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulations (Bronzi, Rosenthal & Gessner 2011). Appendix II includes species that are not necessarily now threatened with extinction but that may become so unless trade is closely controlled (CITES 2010). The only *Acipenser* or *Huso* species listed in Appendix I of the CITES regulations, which lists species that are threatened with extinction and are prohibited for international trade except when the purpose of the import is not commercial, are *A. brevirostrum* and *Acipenser sturio* (CITES 2010). International trade of both wild and commercially reared sturgeon products including caviar, meat and skin requires a CITES permit or certificate issued by a relevant national CITES management authority (CITES 2013; Jahrl 2013). Sturgeon exporters and producers are also required to have a license from a relevant CITES management authority in the country of production. To protect wild stocks there are internationally agreed quotas for trade in sturgeon products. To support transparency in the allocation of sturgeon quotas, all sturgeon production and trade must be recorded (Bronzi, Rosenthal & Gessner 2011; Jahrl 2013). All importing countries must control the

complete supply-chain from unload to transit, repackaging, relabel and re-export activities for all sturgeon products. This resolution also regulates the labelling of wild-caught and farmed caviar (CITES 2013).

Despite the regulations in place, illegal fishing and illegal trading of sturgeon and its products still occurs (Stupachenko 2021; Thuy 2021; van Uhm & Siegel 2016). The main drivers for this are the extremely high value of sturgeon caviar and the demand for sturgeon meat, considered a delicacy in some regions (van Uhm & Siegel 2016; WWF 2021). A survey was conducted by the World Wildlife Fund in 2016–20 on sturgeon meat and caviar collected from restaurants, shops, markets, aquaculture facilities, fishermen or online suppliers in Bulgaria, Romania, Serbia and the Ukraine (WWF 2021). The samples underwent DNA testing and isotope analysis to identify the sturgeon species they were derived from and to assess if the samples were from wild or aquaculture sources. In total, 19% of samples (n=145) originated from wild sturgeon, despite the prohibition of trade on the products in the region, and 12% of samples were not in compliance with international trade regulations, with an absence of mandatory CITES labelling, or correct codes for species or country of origin (WWF 2021). Both whitewashing (selling wild sturgeon products as derived from captive-bred fish) and blackwashing (selling farmed products as derived from wild-caught sturgeon), along with various forms of fraud (meat from other fish sold as sturgeon or artificial products as caviar) were also found (WWF 2021).

There has been a call for global conservation measures including scientific research, population monitoring, law enforcement improvement, and regional cooperation for sturgeon industries (CITES 2013). Examples of conservation activities include DNA and tissue cryopreservation collections, protection of spawning and feeding grounds, and implementation of a fishing licence system (CITES 2000; Gesner, Chebanov & Freyhof 2010). Caspian Sea countries such as the Islamic Republic of Iran, the Russian Federation and Ukraine have also implemented sturgeon sperm-banks for artificial reproduction (Billard et al. 2004; Ovissipour & Rasco 2012).

## 2.3 Sturgeon aquaculture industry

Sturgeon aquaculture was initiated to restock the wild sturgeon population and to satisfy market demand for caviar and food products. However, several sturgeon production systems continue to rely on sourcing fish from the wild to be used as broodstock (Chebanov & Galich 2013). Sturgeon hatcheries began around the same time as bans on sturgeon fishing were implemented, particularly in the Caspian Sea (around 1959–72) (Ruban & Khodorevskaya 2011). The Russian Federation was the first country to develop sturgeon aquaculture, followed by Germany, Italy, France and North America in the 1980s (Williot et al. 2001).

Sturgeon farming for caviar is expensive and requires a significant time investment. This is due to the nature of the fish with its slow growth rate and time to reach maturity. For example, *A. baerii* requires 3–4 years to reach sexual maturity under optimal aquaculture conditions, while *H. huso* requires over 10 years (Gesner, Chebanov & Freyhof 2010; Jahrl 2013; UNODC 2016). Historically, most caviar production was from wild-caught fish. However, since late 2007, aquaculture caviar production has exceeded wild-caught sources (Bronzi et al. 2019).

Ranked by shipment volume, the top exporters of caviar in 2021 were China (244 tonnes), Italy (234 tonnes), United States of America (72 tonnes), Lithuania (51 tonnes), Republic of Türkiye

(42 tonnes), France (33 tonnes), Canada (31 tonnes), Poland (30 tonnes), Maldives (26 tonnes), Latvia (26 tonnes), Germany (25 tonnes), Denmark (24 tonnes), Spain (24 tonnes) and other countries to a total volume of 765 tonnes; although it is not specified what species the caviar is produced from (Workman 2022). In terms of international sales, the biggest exporters of caviar in 2021 were China (US\$63.9 million), Italy (US\$26.9 million), France (US\$21.8 million), Germany (US\$14.1 million), Poland (US\$9.2 million), Belgium (US\$8 million), United States of America (US\$3.5 million), Netherlands (US\$3.23 million), Israel (US\$3.19 million) and other countries to a total of US\$180 million (Workman 2022). Bronzi et al (2019) reported that the species contributing to caviar production in 2016 were dominated by *A. baerii* (31% of the total volume), followed by *Acipenser gueldenstaedtii* (20%), the hybrid *Huso dauricus* × *Acipenser schrenckii* (13%), *A. transmontanus* (12%) and *H. huso* (1%), while other species jointly contributed 23% to the overall yield (Bronzi et al. 2019). Caviar is considered a luxury product aimed at mostly high-income consumers and is one of the most expensive animal products in the world. Prices for caviar can vary widely depending on which sturgeon species the caviar is harvested from and the grading of the product (ABARES 2017). The retail price of *H. huso* caviar can be as high as US\$10,000/kg (UNODC 2016).

In 2017, there were 2,329 commercial sturgeon farms globally although this number is likely an underestimate because reliable data was unavailable for 18% of the countries involved in sturgeon production (Bronzi et al. 2019). Of these farms, 54% were in China (1,254 farms), 24% in the Russian Federation (555 farms), 8% in the Middle East (181 farms), 7% in the Far East (168 farms) and 6% in Europe (143 farms) (Bronzi et al. 2019). Aquaculture sturgeon biomass production in 2017 was estimated at 102,327 tonnes with China producing approximately 79,638 tonnes, followed by the Russian Federation with 6,800 tonnes, Armenia with 6,000 tonnes, Islamic Republic of Iran with 2,514 tonnes and 52 other countries with less than 1,000 tonnes each (Bronzi et al. 2019). Thirteen pure sturgeon species and 4 hybrids were farmed for meat in 2016 with *A. baerii* dominating production with a share of 39.5%, followed by the two hybrids, *H. dauricus* × *A. schrenckii* and *A. baerii* × *A. schrenckii* (35.6%), as well as *A. schrenckii* (10.2%) and *H. huso* (1.35%) (Bronzi et al. 2019). Sturgeon aquaculture production in Europe in 2019 was reported as 2,724 tonnes (EURL for Fish and Crustacean Diseases 2021). World farmgate sturgeon meat prices can vary. In 2022, the price for *Acipenseridae* meat fillets in France was €15 (US\$15.79)/kg and €7.50 (US\$7.89)/kg for gutted sturgeon (FAO 2022b).

Among sturgeon aquaculture rearing technologies, flow-through (FT) systems were most common in 2016 (36% of facilities), followed by RAS (21%), cages (18%), mix FT/RAS (11%) and ponds (6%) (Bronzi et al. 2019). In some countries, sturgeon are commonly reared in earthen ponds as polyculture with other fish species (Patriche et al. 2002; Pyka & Kolman 2003), but there has also been interest in the polyculture of juvenile sturgeon with other fish species in RAS (Dalsgaard et al. 2013; Mihailov et al. 2020; Mihoc et al. 2021; Szczepkowski & Szczepkowska 2006; Thomas et al. 2020). For example, *A. baerii* and *A. gueldenstaedtii* were cultured in the same RAS with *Perca fluviatilis* (European perch, redbin) and *Sander lucioperca* (pike-perch) (Rupp et al. 2019) and *A. transmontanus* has been polycultured with *Oncorhynchus mykiss* (rainbow trout) (LaPatra et al. 1995).

Due to the length of time to maturity (up to 12 years in some species) and value of sturgeon products, there is an industry incentive to maintain a high health status of cultured sturgeon

populations. However, none of the primary viral infections of sturgeon are listed by the World Organisation for Animal Health (WOAH) or European Union legislation, and therefore, there is a lack of regular screening for these diseases (Axen, Vendramin & Toffan 2018). This has resulted in poorly implemented translocation controls and contributed to the spread of disease agents through subclinically infected animals (Axen, Vendramin & Toffan 2018).

### **2.3.1 *Acipenser baerii* (Siberian sturgeon) aquaculture**

*A. baerii* dominates both caviar and meat production. In 2020, *A. baerii* global aquaculture meat production was 442 tonnes (live weight), valued at US\$3.3 million (FAO 2022c). FAO reports countries that farm this species for meat include Belarus, Bulgaria, Cyprus, Spain and Uruguay (FAO 2022a). It should be noted that data may not be available for all sturgeon producing countries from the FAO. According to CITES, 76.7 tonnes of *A. baerii* caviar was traded in 2021 and it was farmed in Austria, Armenia, Belarus, Belgium, Bulgaria, China, Finland, France, Germany, Greece, Hungary, Islamic Republic of Iran, Italy, Latvia, Japan, Madagascar, Netherlands, Poland, the Russian Federation, Spain and Uruguay (CITES 2023). It typically takes at least 7 years for female *A. baerii* to reach sexual maturity and 6 years for males but can take 3–4 years under optimal conditions found in a sturgeon farm (FAO 2023; Jahrl 2013; UNODC 2016).

### **2.3.2 *Huso huso* (Beluga) aquaculture**

*H. huso* typically produce the most expensive caviar. According to CITES, 20.8 tonnes of *H. huso* caviar was traded in 2021 and it was farmed in Azerbaijan, Belgium, Bulgaria, China, France, Germany, Italy, Islamic Republic of Iran, Republic of Moldova and Romania (CITES 2023). FAO reported global *H. huso* aquaculture meat production peaked in 2010 at 115 tonnes, valued at US\$981,000, and in 2020 was 25 tonnes (live weight) valued at US\$188,000 (FAO 2022c). In aquaculture, the male fish typically reach maturity at 5–8 years of age, while females are at maturity around 9–12 years of age (Chebanov & Galich 2013).

### **2.3.3 Potential sturgeon aquaculture industry in Australia**

Live sturgeon or their reproductive material are currently not permitted import into Australia, therefore there is no existing sturgeon meat or caviar industry in this country. All consumption of caviar in Australia is from imports. The value of caviar imports into Australia increased from AU\$2.1 million in 2012 to AU\$3.6 million in 2021 (ABS 2022). According to CITES, Australia imported 2.6 tonnes of caviar in 2020 and 5 tonnes in 2021 (CITES 2023). The caviar was predominantly produced by *A. baerii*, *A. gueldenstaedtii*, *A. transmontanus*, the hybrid *H. dauricus* × *A. schrenckii* and *H. huso* (CITES 2023). Australian imported only 5.6 kg of sturgeon meat in 2021 from *A. baerii* and *A. gueldenstaedtii* (CITES 2023).

The market for caviar in Australia is currently relatively small so domestic producers would initially be highly dependent on the export market to achieve growth in production (ABARES 2017). Australian exporters have access to world markets and would have a competitive advantage in exporting to countries that have a free trade agreement in force with Australia. Australia has free trade agreements with two major world caviar importers, Japan and the United States of America (ABARES 2017). Although some market sectors demand wild products, farmed caviar has similar acceptance as wild origin product (Bronzi et al. 2019).

The Australian sturgeon aquaculture industry can be established and supplemented with imported stock. This can be achieved through the transport of live sturgeon or their reproductive material which can be sent over long distances with close to 100% survival (ABARES 2017). However, the cost structure of a potential Australian-based farm is uncertain, electricity, feed and labour costs are likely to be key cost drivers (ABARES 2017). Further, Australia's high cost of aquaculture production can make competing against low-cost aquaculture-producing countries difficult (Curtotti et al. 2023). This could be addressed by either reducing energy costs through renewable generation, increasing the caviar production from each sturgeon or polyculturing other fish species for fish products to improve early stage cashflow and increase profitability (ABARES 2017; Pyka & Kolman 2003). In Australia, the most like fish species to be polycultured with sturgeon would be salmonids.

## 2.4 Sturgeon aquaculture production practices

Stages in sturgeon aquaculture practices include:

- broodstock selection (particularly for wild stock)
- spawning and egg fertilisation
- grow out (to reach the mature age).

### 2.4.1 Broodstock selection

Sturgeon broodstock may be either sourced from wild fish or domesticated lines. Due to the long time required for sturgeon spawning, wild broodstock are often captured each year for aquaculture purposes (Chebanov & Billard 2001). Wild broodstock are captured using nets and traps in the river mouths and coastal areas during the fish spawning migration season, particularly for species that migrate up river from the sea to spawn such as *H. huso* (Abdolhay 2004; Chebanov & Galich 2013). The migration seasons are usually in spring and autumn months in the Northern Hemisphere (Chebanov & Galich 2013). Although, most wild *H. huso* broodstock are caught during winter (Abdolhay 2004).

Wild broodstock require similar conditions to their natural environment to minimise stress. Fish need to be acclimated for around 2–3 months in specific conditions including natural photoperiod, low water temperatures (10–15°C), high oxygen level and low stocking density. Earthen or concrete ponds and cages are commonly used as wild broodstock holding units (Chebanov et al. 2011). During this acclimatisation process, the fish are monitored for any sanitary, health or behavioural issues (Chebanov et al. 2011; Edwards & Doroshov 1989).

Typical sturgeon hatcheries have separate units for holding pre-spawning broodstock, spawning broodstock, gamete collection, larvae rearing tanks, fry rearing tanks as well as facilities for water treatment and producing live food (Chebanov & Galich 2013).

### 2.4.2 Spawning and egg fertilisation

Female sturgeon do not ovulate every year. For example, usually only 35–63% of the *A. baerii* mature female cohort from one stock ovulate annually (FAO 2005). Sturgeon eggs for aquaculture purposes are collected from female broodstock using non-lethal surgical methods (Mohler 2003).

Once eggs have been collected and enumerated, they are fertilised with milt as soon as possible, followed immediately by de-adhesion and disinfection procedures (Mohler 2003). The eggs are

typically placed into incubation devices for hatching (Mohler 2003). Once the larvae have hatched they are moved into receiving tanks for rearing (Mohler 2003).

### **2.4.3 Grow out**

Fry that have outgrown their rearing tanks can be transferred for grow out into a wide variety of systems such as raceways, tanks, RAS, ponds and net cages (Chebanov & Galich 2013). Different types of freshwater systems are used for enclosed aquaculture including surface water, well water (including geothermal) and industrial wastewaters (including thermal effluents). It is noteworthy that although sturgeon spend some of their life in seawater they are reared completely in freshwater (Williot et al. 2001). Several technologies have been used to treat, sterilise and disinfect water for grow out, including ozonation, ultraviolet light, temperature control (heating), acidity neutralisation, suspended solid removal (sediment trap and filter) and iron oxidation with subsequent settling of the oxides (Chebanov et al. 2011).

Sturgeon are temperate fish with the optimal water temperature for culture being between 15–20°C (Castellano et al. 2017; Mohler 2003) but have a wide tolerance for low (1°C) and high (30°C) water temperatures depending on the species (USGS 2011). Culturing sturgeon at higher water temperatures is usually avoided as this has been shown to negatively affect the stress levels, growth rate and innate immune defences of sturgeon (Castellano et al. 2017).



### 3 Finfish industries in Australia

The gross value of Australia's finfish production in 2020–21 was estimated at AU\$1.76 billion from a total weight of 227,002 tonnes (Tuynman & Dylewski 2022). The aquaculture industry produced 103,833 tonnes valued at AU\$1.26 billion while wild-caught fisheries produced 127,763 tonnes with an estimated value of AU\$503 million (Tuynman & Dylewski 2022).

Fisheries and aquaculture based in state and territory waters produced 89% of total fisheries production for 2020–21 (Tuynman & Dylewski 2022). 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 Australian waters (AFMA 2022).

#### 3.1 Aquaculture production

In Australia, the main finfish aquaculture species in 2020–21 were salmonids (including *Salmo salar* (Atlantic salmon) and trout), *Thunnus maccoyii* (Southern Bluefin Tuna) and *Lates calcarifer* (barramundi). There is also aquaculture of other fish species such as *Anguilla australis* (shortfin eel), *Anguilla reinhardtii* (longfin eel), *Bidyanus bidyanus* (silver perch), *Maccullochella peelii* (Murray cod), *Macquaria ambigua* (golden perch), *Macquaria novemaculeata* (Australian Bass), *Scortum barcoo* (Jade perch), *Seriola lalandi* (yellowtail kingfish) and ornamental fish species (Tuynman & Dylewski 2022).

##### 3.1.1 Salmonids

Salmonids are the predominant finfish species cultured in Australia with an estimated 84,341 tonnes produced in 2020–21 with a value of AU\$1.03 billion (Tuynman & Dylewski 2022). Approximately 98% of Australia's salmonid aquaculture production is in Tasmania (83,056 tonnes) with 1.7% produced in Victoria (989 tonnes) and the remainder in South Australia and Western Australia (296 tonnes) (Tuynman & Dylewski 2022). Salmonid species currently commercially farmed in Tasmania include *S. salar* and *Oncorhynchus mykiss* (rainbow trout) (Tasmania 2022).

In Australia, early-stage salmon are farmed in freshwater facilities and after 12–18 months when they become smolt (a development stage where they become able to tolerate seawater) the fish are transferred to cages located in estuaries or sheltered coastal waters. Some pre-smolts are transferred to brackish water as a prelude to transfer to full seawater. *S. salar* grow from around 80 g to a marketable size of 3.4–4.5 kg in 12–15 months after introduction to saltwater. This is partly attributable to the relatively warm waters in which Australian salmon are farmed. Most Australian salmon producers harvest fish all year round. Currently, the main salmonid producers in Tasmania are Tassal, Huon and Petuna (Tasmanian 2022).

##### 3.1.2 Southern Bluefin Tuna

In 2020–21, Southern Bluefin Tuna aquaculture production in South Australia was estimated at 7,600 tonnes valued at AU\$91 million (Tuynman & Dylewski 2022). Southern Bluefin Tuna are captured live and then transferred to aquaculture farming operations off the coast of Port Lincoln where they are grown out to reach market size (Patterson et al. 2022).

### 3.1.3 Barramundi

Barramundi aquaculture produced an estimated 7,815 tonnes in 2020–21 with a value of AU\$86 million (Tuynman & Dylewski 2022). This volume is likely an underestimate due to some data not being available. The Australian Barramundi Farmers Association (ABFA) reports barramundi aquaculture production in 2019–20 was 9,000 tonnes valued at AU\$90 million (ABFA 2022). Barramundi is cultured in both freshwater and marine environments using land-based ponds, raceways, RAS and net cages (Harrison, Calogeras & Phillips 2014). The ABFA reports that barramundi is farmed in all mainland states and the Northern Territory (ABFA 2022). In 2020–21, ABARES reports production in Northern Territory (3,665 tonnes valued at AU\$39 million), Queensland (3,478 tonnes valued at AU\$35 million), Victoria (615 tonnes valued at AU\$11 million) and New South Wales (57 tonnes valued at AU\$759,000), although it is mentioned that barramundi is also produced in South Australia and Western Australia (Tuynman & Dylewski 2022). ABFA anticipates a major increase in production in the next 3 years, with a national production target of 20,000 tonnes by 2025 (ABFA 2022). Two of Australia’s largest barramundi producers are Humpty Doo Barramundi in the Northern Territory and MainStream Aquaculture in Victoria (Norwood 2019).

## 3.2 Fisheries

Wild-caught finfish products were valued at AU\$503 million in 2020–21 (Tuynman & Dylewski 2022). Tuna is the largest and single most valuable wild-caught finfish species in Australia and are largely caught in Commonwealth fisheries (AFMA 2022). In 2020–21, at least 9,283 tonnes of tuna was caught in Australia valued at AU\$66 million (Tuynman & Dylewski 2022). Other wild-caught species include Australian sardines (AU\$30 million), coral trout (AU\$26 million), flathead (AU\$25 million), whiting (AU\$16 million), barramundi (AU\$9 million) and other unspecified finfish (AU\$243 million) (Tuynman & Dylewski 2022). Value and quantity data is not available for some wild-caught fisheries due to confidentiality restrictions (Tuynman & Dylewski 2022). The extensive list of finfish species wild-caught in Australia in 2020–21 is available in the ABARES [Australian Fisheries and Aquaculture Statistics report](#) (Tuynman & Dylewski 2022) and [Fishery status reports 2022](#) (Patterson et al. 2021).

## 3.3 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).

Recreational fishing activities may be divided into freshwater and saltwater activities. Nationally, 23.8% of the fishing activities occurred in saltwater (offshore, coastal and estuarine waters), 3.4% in freshwater (freshwater rivers, lakes and dams) and 44.4% in both saltwater and freshwater (Moore et al. 2023).

In the 2019–20 Queensland recreational fishing survey, whiting were reported as the most harvested fish followed by yellowfin bream, mullet and coral trout (DAF 2020). The survey of recreational fishing in New South Wales and the ACT 2013–14 lists bream, flathead, snapper, whiting and European carp among the top species caught (West et al. 2015). In Victoria, a web-based survey conducted in 2012 identified the preferred catch species in rivers and lakes were rainbow trout,

brown trout and redfin and in estuaries were bream and flathead (VFA 2012). The 2021–22 recreational fishing survey conducted in South Australia reported that King George Whiting was the most caught marine fish followed by Australian herring, Southern garfish and Western Australian salmon (Beckmann et al. 2023). In freshwater, the most caught fish species were carp, golden perch and Murray cod (Beckmann et al. 2023). The 2017–18 survey of recreational fishing in Tasmania found flathead had the highest catch by number and then trout, Australian Salmon, gurnards and wrasse for marine species whereas Atlantic salmon and redfin perch were high catch species in freshwater (Lyle et al. 2019). For the 2020–21 recreational fishing survey conducted in Western Australia, the most popular fish caught state-wide were school whiting, Australian herring, pink snapper and King George Whiting (Ryan, Lai & Smallwood 2022). In the survey of recreational fishing in the Northern Territory 2018–19, golden snapper was the most frequently caught fish species in offshore and inshore waters, along with tropical snapper, rockcod/grouper, longtail and mackerel tuna (West et al. 2022). Barramundi dominated catches in estuarine waters followed by golden snapper, catfish and mullet and in freshwater the main species caught were barramundi, oxeeye herring, catfish and bream (West et al. 2022).

Several states and territories release fish into natural waterways as part of ongoing fish stocking programs to improve recreational anglers' fishing experience. For example, South Australia periodically releases rainbow trout, Murray cod, golden perch and silver perch (Water 2023). The same fish are released into Victoria waterways along with brown trout, Chinook salmon and Australian bass, (VFA 2022).

### 3.4 Ornamental fish

In 2020–21, the estimated aquaculture production of ornamental fish was AU\$1.2 million in Queensland (volume not available), AU\$655,000 in New South Wales (volume not available), AU\$631,000 in Victoria (volume not available), AU\$506,000 in Western Australia (volume not available) and also occurred in the Northern territory (value and volume not available) (Tuynman & Dylewski 2022). In addition to commercial breeders, ornamental fish are also bred by wholesalers, retailers and the hobby sector, which is difficult to quantify. Ornamental fish species commercially bred include *Carassius auratus* (goldfish) (Tuynman & Dylewski 2022).

### 3.5 Australia's trade in finfish

#### 3.5.1 Finfish imports

The value of imported finfish, shark and ray products in 2020–21 was AU\$1.25 billion (152,482 tonnes). The major countries exporting fishery products to Australia in 2020–21 were Thailand, Vietnam, China and New Zealand (Tuynman & Dylewski 2022). No live imports of finfish for commercial aquaculture or human consumption have been permitted into Australia for over 50 years. However, Australia's largest finfish aquaculture industry is based on *S. salar*, introduced from England and the Americas in the late 1800's (Clements 1988). Live ornamental fish with an estimated value of AU\$6.9 million (volume not available) were imported in 2020–21 (Tuynman & Dylewski 2022). It must be noted that under the [Environment Protection and Biodiversity Conservation Act 1999](#), the import of *Acipenser baerii* and *Huso huso* is only permitted for commercial aquaculture. Sturgeon cannot be imported to Australia as ornamental fish.

### **3.5.2 Finfish exports**

In 2020–21, Australia exported 24,470 tonnes of salmonids (AU\$259 million) and 12,232 tonnes of tuna (AU\$144 million). An additional 11,215 tonnes of finfish, shark and ray products (from both aquaculture and wild fisheries sectors) valued at AU\$106.8 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 & Dylewski 2022). In 2020–21, AU\$1.5 million of live ornamental fish were also exported (volume not available) (Tuynman & Dylewski 2022).

## 4 Method

The World Organisation for Animal Health (WOAH), in its *Aquatic animal health code* (WOAH Code), describes 'General obligations related to certification' in chapter 5.1 (OIE 2021a).

The WOAH Code states in Article 5.1.2. 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. 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.

The four components of risk analysis as described in chapter 2.1. of the WOAH Code are:

- hazard identification
- risk assessment (entry, exposure and consequence assessment 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.

Sources of information drawn on for this draft biosecurity import risk analysis (BIRA) include (this list is not exhaustive):

- the 1999 *Import risk analysis on non-viable salmonids and non-salmonid marine finfish* (AQIS 1999b)
- the 1999 *Import risk analysis on live ornamental finfish* (AQIS 1999a)
- the 2014 *Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses: Final import risk analysis report* (Department of Agriculture 2014)
- the 2023 *Review of the biosecurity risks of prawns imported from all countries for human consumption-final report* (DAWE 2020).

- the WOA Code (OIE 2021a)
- the WOA *Manual of diagnostic tests for aquatic animals* (OIE 2021f)
- a review of relevant scientific literature
- opinion of subject matter experts.

## 4.1 Hazard identification

Hazard identification is described in the WOA Code (Article 2.1.2) as a classification step that is undertaken to identify potential hazards that may be associated with the importation of a commodity (OIE 2021a). A hazard is a disease agent with the potential for harm.

In accordance with the WOA Code, a disease agent was considered a hazard relevant to the importation of live sturgeon or their reproductive material if it was assessed to be:

- 1) a known disease agent of sturgeon (*Acipenser* and *Huso* species only)
- 2) WOA listed, an emerging disease, or if it can produce adverse consequences in Australia
- 3) not known to be present in Australia, or
- 4) present in Australia, and a notifiable disease, and subject to an official control or eradication program.

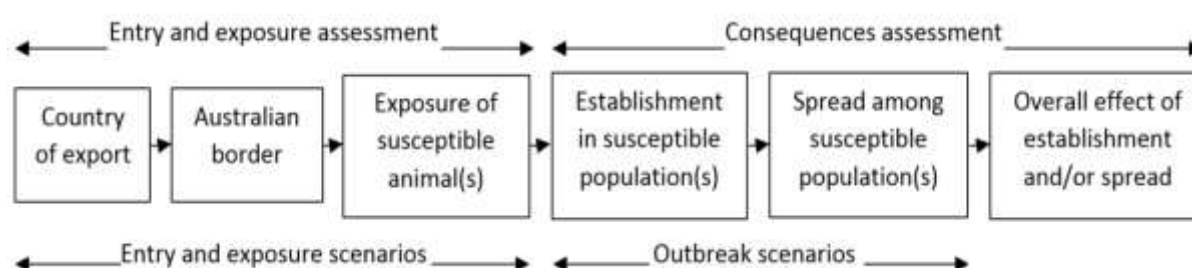
Where evidence for the inclusion or exclusion of a disease agent was equivocal, a judgement was made based on the strength of the available evidence to implicate sturgeon in disease transmission.

The identity of any disease agent should be clearly defined to ensure that the assessment is being performed on a distinct agent, and that biological and other information used in the assessment is relevant to the agent in question. If this is not possible because the causal agent of particular symptoms has not yet been fully identified, then it should have been shown to produce consistent symptoms and to be transmissible.

## 4.2 Risk assessment

Details of the risk assessment process are provided in chapter 2.1 of the WOA Code (OIE 2021a).

In accordance with the WOA Code, the entry assessment estimates the likelihood of entry of each of the hazards into an importing country. The exposure assessment describes the biological pathways necessary for exposure of susceptible animals and estimates the likelihood of exposure occurring. The consequence assessment describes the potential consequences of a given exposure and estimates the likelihood of establishment and spread, and the overall effect/impact of establishment and spread. The unrestricted risk estimate is the combination of the likelihood of entry, exposure and consequence assessments. The steps in determining the unrestricted risk estimate are shown in Figure 1.

**Figure 1 Steps in the risk assessment process**

A review of scientific literature was conducted and contact with relevant experts sought, where necessary, for each hazard retained for risk assessment. Assumptions and judgements that were made in drawing conclusions for each hazard were documented in the relevant risk assessment chapters. Based on this information, a decision was made whether or not to continue with the risk assessment for the relevant hazard.

#### 4.2.1 Evaluating and reporting likelihoods

Likelihood estimations made in this risk 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 BIRA used a qualitative approach. The likelihood of entry, exposure or establishment and spread occurring was evaluated and reported using qualitative likelihood descriptors as described in Table 4.

**Table 4 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. For example, the ability of the hazard to be present in subclinical animals is a key factor in determining the likelihood of the hazard entering Australia in a consignment of sturgeon.

#### 4.2.2 Entry Assessment

The entry assessment considered an unrestricted single-entry scenario defined as the importation of live sturgeon or their reproductive material (including any associated wastes) from all countries for aquaculture purposes into land-based semi-open aquaculture systems. It is considered that this scenario represents the highest biosecurity risk.

The entry assessment considered the predicted volume of product to be imported during a one-year period. Based on expected imports from a prospective sturgeon farmer, it is estimated that 50,000–100,000 eggs/year/farm will be imported into Australia over 2–3 consignments.

Several factors were taken into account in determining the likelihood of a hazard entering Australia in imported live sturgeon or their reproductive material, including:

- the biological characteristics of the hazard in live sturgeon or their reproductive material
- likelihood of detection of infected live sturgeon or reproductive material before export
- ability of the hazard to remain infectious through transport.

The absence of a hazard from a region is an important consideration in an entry assessment. The scope of this BIRA includes importation of live sturgeon or their reproductive material from all countries. Therefore, the entry assessment assumes that the hazards are present in all source countries. The amount of a hazard in live sturgeon or their reproductive material exported to Australia depends on many factors, including the biology of the hazard and the production system. Country, zone or compartment freedom from hazards is considered as a biosecurity measure during risk evaluation (see [Sourcing from disease-free stocks](#)).

### **Biological characteristics of the hazard in live sturgeon or their reproductive material**

There are several biological factors of the hazards that are considered during the entry assessment.

#### *Life cycle stage*

The prevalence of infection and/or the expression of disease may vary with the life cycle stage of the host. For example, sturgeon nucleocytoplasmic large DNA virus (sNCLDV) outbreaks are most common when sturgeon are in the juvenile stage (Hedrick et al. 1990; Kurobe et al. 2011; Mugetti et al. 2020b). Fish that survive disease outbreaks at one life cycle stage can become carriers of infection at later life cycle stages. Such is the case with infectious haematopoietic necrosis virus (IHNV), where *Oncorhynchus nerka* (sockeye salmon) and *Oncorhynchus mykiss* (rainbow trout) survivors remained infected with IHNV months to over one-year post-exposure (Drolet et al. 1995; Muller et al. 2015).

#### *Production system*

The production system, husbandry techniques and health management employed in the farms/aquaculture facilities can have a significant influence on the health status of sturgeon. Fish produced in extensive systems with low stocking densities typically have a lower prevalence of disease. This is presumably due to less efficient transmission of disease agents and greater resistance to infection due to lower stress levels. For example, in an experimental study with white sturgeon iridovirus (WSIV), higher cumulative mortalities occurred in *Acipenser transmontanus* reared at higher densities compared to lower densities (Drennan et al. 2005). The variation in mortality was attributed to the higher stress when sturgeon were stocked at higher densities (Drennan et al. 2005).

### **Likelihood of detection of infected live sturgeon or reproductive material before export**

Exporters would aim to send live sturgeon or reproductive material of good quality because high mortality or morbidity in a consignment would lead to loss of future trade. Live sturgeon that are obviously diseased and showing clinical signs would therefore be unlikely to be exported. However, if the exporter has no concern about losing a portion of the sturgeon and there are no pre-export inspection requirements then infected sturgeon might be imported to Australia.

In some cases, disease agents can be carried by live sturgeon not showing clinical signs of disease or only showing mild clinical signs. Certain disease agents may also be difficult to detect before export. For example, low-level infestations of *Argulus species* typically cause no clinical signs in fish and



infestations of small, young ectoparasites can go unnoticed (Steckler & Yanong 2012). As a result, infected live sturgeon may not be detected before export and could be included in consignments imported into Australia. Infected or contaminated reproductive material is also likely to go undetected as they typically will not show any clinical signs of infection. The probability of consignments containing infected live sturgeon or reproductive material would be expected to reflect the prevalence of the disease agent in the country, compartment or zone which the population was sourced from.

#### **Ability of the hazard to remain infectious through transport**

It is expected that the transport of live sturgeon would follow a similar process to that used to transport live ornamental fish to Australia. This process is described in the *2014 Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses Final import risk analysis report* (Department of Agriculture 2014). The procedure for transporting sturgeon eggs is likely similar to that recommended for salmonid eggs (SJRRP 2016). For both live fish and eggs, hazards are likely to survive during transport since the primary conditions for their survival are fulfilled by presence in or on a live susceptible host.

Milt could be transported either fresh at cold temperatures or frozen, although frozen would be more practical in ensuring viability during any travel delays and for storage on arrival (Billard et al. 2004; Chebanov et al. 2011). Most viruses of fish and some bacteria can remain viable at cold temperatures for hours to days (Jakobsen et al. 2020). Viruses are typically able to survive freezing, whereas bacteria are often inactivated to some degree by freezing (Burke & Mulcahy 1983).

Transport water and packaging material can also carry disease agents. Several disease agents such as *A. salmonicida salmonicida* can survive outside the fish host for extended periods (Hiney et al. 2002). It is therefore necessary to consider the probability that material imported with live sturgeon may be vectors for disease agents.

#### **Estimation of likelihood of entry**

The entry assessment is the estimated annual likelihood of each hazard entering Australia in imported live sturgeon or their reproductive material. The entry assessment used the qualitative likelihood descriptors described in Table 4.

The outcome of the entry assessment was an annual likelihood of entry (LR) into Australia of the hazard in imported live sturgeon or their reproductive material.

### **4.2.3 Exposure assessment**

The exposure assessment determines the likelihood that a susceptible population (exposure group) in Australia will be directly exposed to the hazard present in the imported live sturgeon or their reproductive material (including any associated wastes). It considers the exposure groups most likely to be affected as well as the possible pathways by which this could occur. All estimates of the likelihood of exposure assume the hazard is present in the imported live sturgeon or their reproductive material at the time of arrival in Australia.

The factors considered when estimating the likelihood of an exposure group encountering a hazard and establishing as an index case, for each exposure pathway (from entry into Australia, through transport, being farmed and any associated waste disposal), included the:

- likelihood of imported live sturgeon or their reproductive material entering the general environment of the exposure groups
- likelihood of contact between imported live sturgeon or their reproductive material and susceptible host animals
- amount of infectious hazard material in imported live sturgeon or their reproductive material at point of exposure.

#### **Identification of exposure groups**

The term 'exposure group' categorises a group of species that may be susceptible to one or more of the hazards considered in the risk assessments. The two exposure groups considered most likely to be exposed to imported live sturgeon or their reproductive material are:

- farmed susceptible species (including broodstock, eggs, fry, juveniles and adults)
- wild susceptible species.

The farmed susceptible species exposure group includes species grown for human consumption, release into the wild, or export.

There are also populations of fish within the ornamental fish industry (which includes ornamental fish wholesalers, retailers, hobbyists, and public aquariums) and zoos in Australia. However, these exposure groups were not included in the BIRA as sturgeon can only be imported into Australia under the EPBC Act for commercial aquaculture. Sturgeon also have significant housing requirements due to their large size and the likelihood of a sturgeon being diverted and entering the ornamental fish industry is considered unlikely. It is known that in some countries, such as the United Kingdom, there is an ornamental trade in sturgeon and there are problems with subsequent illegal stocking in the wild (Britton & Davies 2006). However, as import and diversion of sturgeon to the ornamental fish trade in Australia is associated with an illegal pathway it is not considered within the scope of the BIRA.

#### **Identification of exposure pathways**

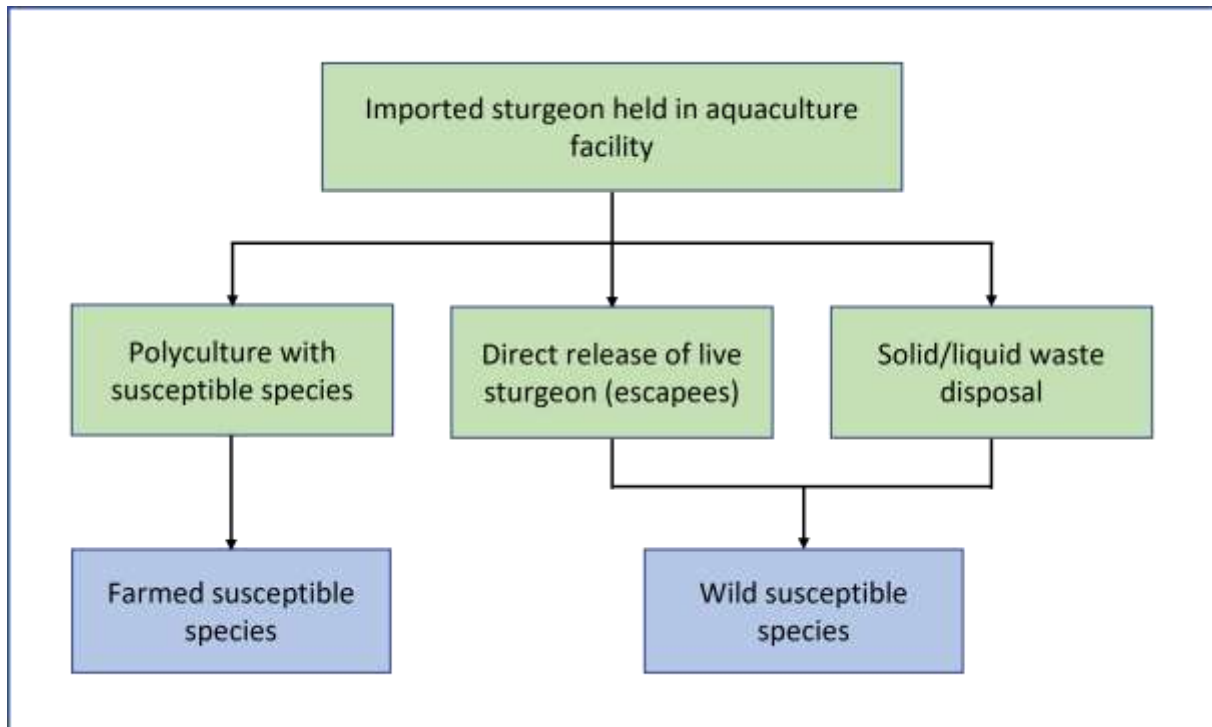
The exposure assessment considers the key pathways and end-uses that may result in the exposure groups encountering each hazard. It takes into consideration that the imported live sturgeon or their reproductive material will only be used for commercial aquaculture.

The pathways identified as contributing to the total risk were:

- imported live sturgeon polycultured with susceptible species in the same aquaculture facility
- direct release of imported live sturgeon (deliberate or accidental) into natural waters
- discharge of water and waste from sturgeon farms into natural waters.

Figure 2 shows the three most likely exposure pathways by which the two exposure groups could be exposed to imported live sturgeon or their reproductive material in Australia. The illegal holding of imported sturgeon in facilities other than those for commercial aquaculture and the possible exposure to farmed and wild susceptible species was considered outside the scope of this BIRA.

**Figure 2 Potential exposure pathways of susceptible populations in Australia to imported live sturgeon or their reproductive material**



**Likelihood of imported live sturgeon or their reproductive material entering the general environment of the exposure groups**

The likelihood of imported live sturgeon or their reproductive material entering the general environment of the exposure groups was estimated for the pathways that substantially contribute to the total risk.

*Imported live sturgeon polycultured with susceptible species in the same aquaculture facility*

Imported live sturgeon held in commercial aquaculture facilities may be co-cultured with sturgeon already present in the facility or polycultured with other susceptible species. Therefore, exposure of farmed susceptible species to a hazard may occur at some stage of their life cycle. For example, *Acipenser iridovirus*-European (AcIV-E) was transmitted from infected *Acipenser gueldenstaedtii* to healthy *Acipenser ruthenus* and *Acipenser stellatus* reared on the same farm (Mugetti et al. 2020a). Also, it was demonstrated that *A. salmonicida salmonicida* could be transmitted from infected *Salvelinus fontinalis* (brook trout) to *Acipenser oxyrinchus* by cohabitation resulting in clinical disease and mortalities (Mohler 2003). A trial in Republic of Türkiye showed polyculture of *Acipenser baerii* with *Oncorhynchus mykiss* (rainbow trout) was possible (Ak et al. 2019). Both *S. fontinalis* and *O. mykiss* and other salmonid species are present in Australia and are susceptible to several of the hazards such as *A. salmonicida salmonicida*, IHNV and *Yersinia ruckeri* (Hagerman strain) (Australian Government Department of Agriculture 2019). Other finfish species present in Australia that have been polycultured with sturgeon are *Cyprinus carpio* (common carp) (Mihailov et al. 2020; Patriche et al. 2002) and *Perca fluviatilis* (European perch, redfin) (Rupp et al. 2019).

It is assumed that due to the stressors associated with intensive aquaculture (e.g. higher density of susceptible animals and culture conditions) that farmed susceptible species are generally more

conducive to hazard exposure compared to wild susceptible species (Department of Agriculture 2014).

*Direct release of imported live sturgeon into natural waters*

All state and territory governments have legislative controls on aquaculture production, including the rearing of fish in secure recirculating aquaculture systems (RAS), net cages, tanks and open ponds. Aquaculture operations are required to be licensed and approval must be obtained from regulatory agencies on various management practices, including to manage and control for fish escapes. Despite this, fish escapes can and do still happen although more often from net cages rather than open ponds (Naylor et al. 2005). Large introductions of exotic sturgeon species into German, Polish and Dutch coastal waters occurred due to accidental fish releases or escapes from aquaculture facilities as a consequence of technical defects (Arndt, Gessner & Raymakers 2002; Gessner et al. 1999). Escape of imported live sturgeon from aquaculture facilities near or directly connected to natural waters, either deliberately or inadvertently, may expose wild susceptible species to imported sturgeon and any hazards they might be carrying. The likelihood of this occurring is expected to be low due to the biosecurity measures that would be in place to reduce fish escaping in licenced operations. However, this level of risk reduction is based on industry compliance, the biosecurity requirements specified in the legislation and the enforcement of the legislation by the states and territory authorities. Although not an example of escaped fish from aquaculture causing disease in the wild, the deliberate release of common carp from aquaculture facilities in the United Kingdom into the wild for restocking purposes has been speculated as causing the spread of koi herpesvirus (KHV) into the wild (Haenen et al. 2004).

*Discharge of water and waste from sturgeon farms into natural waters*

Water and waste discharge from farms holding infected imported sturgeon into natural waters represents a potentially significant pathway by which hazards could spread to wild susceptible populations. Aquaculture operations in Australia are required to obtain approval from regulatory agencies on various management practices, including water and waste disposal methods. These legislative controls may reduce the likelihood of any infected solid or liquid waste from entering natural waters. The level of risk reduction however is based on industry compliance, the biosecurity requirements specified in the legislation and the enforcement of the legislation by the relevant state or territory authority. For example, following an outbreak of white spot disease on a prawn farm on the Logan River in South-East Queensland, Australia in late-2016, the disease was detected in the wild and neighbouring farms despite legislative controls being in place (Department of Agriculture 2020b). Further, it was noted by the department during its investigations that biosecurity measures on some farms were lacking at that time (Department of Agriculture 2020b).

**Likelihood of contact between imported live sturgeon or their reproductive material and susceptible host animals**

In an unrestricted situation, imported live sturgeon may share water, equipment, tanks or ponds with other sturgeon cohorts or susceptible species present in the aquaculture facilities. For this reason, it is highly likely that farmed susceptible species would be exposed to imported live sturgeon or reproductive material and any hazards they might be carrying. Hazards may be transmitted via fish to fish contact, ingestion of infected host tissue, free hazard in the water column or fomites.

The probability of wild susceptible species encountering either imported live sturgeon or water and waste released from a farm and consequently any hazards, depends on several factors. These factors

include the volume of fish or associated waste released into the natural environment, how long the sturgeon will survive in the natural environment, the dispersal and dilution of the fish or associated waste, the ability of the hazard to survive outside a host and the presence and concentration of susceptible species in the area. Wild susceptible species would be less abundant than susceptible species in aquaculture facilities. For hazards with a wide host range such as frog virus 3 (Chinchar, Waltzek & Subramaniam 2017), the likelihood of wild susceptible species encountering the hazard is greater in comparison to those hazards with a smaller host range such as KHV (OIE 2021c).

There are no specific regulations or codes of practices to control the movement of amphibians or reptiles into commercial aquaculture facilities, although some measures are in place to manage predators such as water rats and birds. If facilities holding imported live sturgeon are not biosecure to the external environment, then there is the potential for wild amphibians to enter and cohabit in the same waters and become exposed to hazards.

#### **Amount of infectious hazard material in imported live sturgeon or their reproductive material at point of exposure**

The amount of infectious hazard present will depend on numerous factors including the tissue titre and disease agent stability.

##### *Amount of hazard at the point of exposure*

Tissue titre refers to the amount of hazard present in a specific tissue type or location in the host. The tissue titre of a hazard has the potential to affect the likelihood of exposure because an infectious dose needs to be present in order to infect a susceptible species. For example, the viral titre in IHNV-infected fish with clinical signs averages  $10^4$ – $10^6$  PFU/g and can be up to  $10^8$ – $10^9$  PFU/g (Dixon et al. 2016), which means one infected fish could contain multiple infectious doses if it is assumed to be at least  $1 \times 10^4$  PFU/mL as shown in experimental challenges (Purcell et al. 2010).

The effect of dilution on the hazard is an important consideration when determining whether a dose sufficient to cause infection in a susceptible host animal would remain should there be an exposure. For example, treatment of effluent water from aquaculture facilities by settlement, dilution and screening before it is released into natural waters could reduce the amount of hazard (or dose) encountered by a wild susceptible species. This settlement process will also reduce the likelihood of escapees. However, the effect of these processes would be less in cases where water effluents are not treated regularly, or significant quantities of effluent water and potentially infected animals are released to the environment before a biosecurity response is enacted in response to the detection of a hazard. Also, if there was an accidental release of many infected fish from a farm then the effect of dilution under this circumstance would be less.

##### *Ability of hazards to remain infectious at point of exposure*

The hazards are highly likely to survive within the imported live sturgeon or their reproductive material due to their live host. Some hazards, such as Missouri River iridovirus (MRIV), can persist and maintain infectivity in live sturgeon for extended periods (Kurobe et al. 2011). The ability of a hazard to remain infectious when in water for extended periods is also an important consideration. For example, *Ergasilus sieboldi* can remain viable in water for up to 10 weeks under certain conditions (Abdelhalim, Lewis & Boxshall 1991). Water temperature can also play a role as some hazards have a narrow range of virulence. For example, IHNV infection typically occurs at water temperatures of 3–18°C (WOAH 2022b).

### Estimation of partial likelihood of exposure

The likelihood that each exposure group would be exposed to a hazard through contact with imported live sturgeon or their reproductive material 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 4).

### 4.2.4 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 two exposure groups.

The PALEE for each exposure group was estimated by combining the likelihood of entry (LR) (see section 4.2.2 [Estimation of likelihood of entry](#)) and the corresponding partial likelihood of exposure (PLE) (see section 4.2.3 [Estimation of partial likelihood of exposure](#)) using the matrix shown in 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

### 4.2.5 Consequence assessment

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

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.

### Identification of the outbreak scenario

Once exposure of a susceptible population to a hazard has occurred, a number of possible outbreak scenarios could follow. These represent a continuum ranging from no spread to establishment and spread of the disease to its natural geographic limits.

For this BIRA, one outbreak scenario was assessed because it has the most potential to occur with significant consequences:

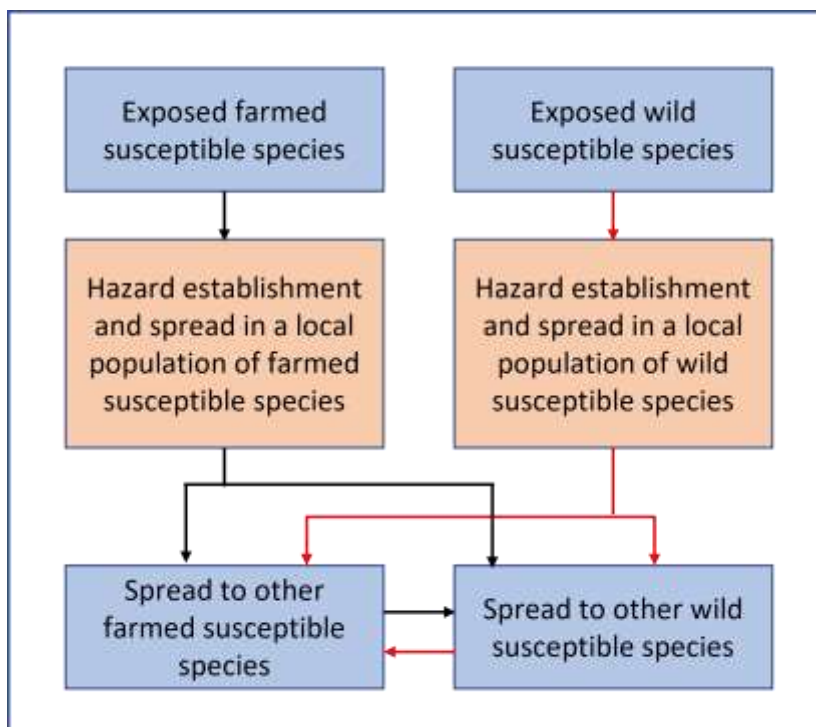
The hazard establishes in the directly exposed population and spreads to farmed and wild populations, is not eradicated, becomes endemic in Australia and eventually spreads to its natural geographical limits.

This is consistent with other risk assessments conducted by the department whereby only one outbreak scenario is assessed (e.g. *Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses—final import risk analysis report* (Department of Agriculture 2014) and *Review of the biosecurity risks of prawns imported from all countries for human consumption* (DAWE 2020)).

It is generally acknowledged that eradication of an aquatic animal disease in the wild is not feasible. If an aquatic disease does establish in a wild population, it is very difficult to prevent its spread by natural means. This is because the disease agent can infect susceptible species to a large extent before authorities can detect and respond with effective control and eradication programs.

It was assumed that a hazard would establish and spread through to its natural geographical limits via the various pathways shown in Figure 4.

**Figure 4 Establishment and spread pathways**





### **Partial likelihood of establishment and spread associated with the outbreak scenario**

The factors considered relevant when estimating the 'partial likelihood of establishment and spread' (PLES) were:

- infectious dose
- mechanisms of spread and transmission
- susceptibility of Australian species to infection.

#### *Infectious dose*

The likelihood that a hazard will establish and spread is affected by how easily the hazard can be transmitted from an index case to other susceptible animals. This is influenced by the dose of the hazard necessary to cause infection and the likelihood that susceptible animals would be exposed to that dose and that the disease/hazard would establish and spread to other hosts. For some hazards, data is available describing a minimum infectious dose. For example, undiluted furuncle material from *A. salmonicida salmonicida*-infected fish can contain  $10^{10}$  CFU/mL bacteria and ingestion of only 1  $\mu$ L ( $10^8$  CFU) by a susceptible fish would be sufficient to establish an infection assuming a minimum infectious dose of  $>10^5$  CFU/fish (Rose, Ellis & Munro 1989). Hazards which have a low minimum infective dose will be more capable of spreading, even in cases of large areas with dispersed susceptible animals. Those hazards which have higher minimum infectious doses will be less capable of establishing and spreading.

#### *Mechanisms of transmission and spread*

The greater the population density of host animals susceptible to disease, the more readily disease may be transmitted, resulting in higher morbidity and increased likelihood of disease agent establishment. Additionally, other factors that affect the susceptibility of the host to infection (e.g. life cycle stage, the health and immunological status of the host, environmental conditions and stress) may also affect transmission.

For most disease agents of sturgeon with a direct life cycle, transmission from an index case(s) to other susceptible species may occur through exposure to free hazard in the water column. For example, *A. salmonicida salmonicida* can survive in freshwater and seawater from 2–63 days and 2–24 days, respectively (Hiney 1994). Vectors and carrier hosts may play a role in the mechanical spread of disease agents through the water. Marine plankton, protozoa and other ectoparasites such as copepods (e.g. salmon lice), are considered possible vectors of *A. salmonicida salmonicida* (Nese & Enger 1993). Fomites may also play a role in the transmission of the disease agent when objects, such as aquaculture equipment, are exposed and then used in other aquaculture establishments or the natural environment, or vice versa. Ingestion of infected host tissues is also a possible transmission pathway. Transmission from broodstock to progeny has been suggested for some disease agents and may occur via infection of the eggs, via contamination of the external surface of the egg, or via release of the disease agent during spawning (e.g. *Yersinia ruckeri* (Barnes et al. 2016)). Some disease agents may cause subclinical infection or can persist in survivors' post-infection, so apparently healthy fish may still be a source of infection.

The dispersal of hazards can occur via several pathways. In the wild, disease agents are typically dispersed by the movement of live hosts, including during natural migration. The movement of infected reproductive material, larvae, fry and juveniles from hatcheries to other aquaculture



facilities or to the wild for restocking has facilitated national and international spread of disease agents. For example, the movement of *Acipenser stellatus* from the Caspian Sea into the Aral Sea introduced the protist gill parasite *Nitzschia sturionus* which is speculated to have caused disease and the substantial decline of the native *Acipenser nudiiventris* (ship sturgeon) (Bauer, Pugachev & Voronin 2002).

Spread of hazards between and within farms (including between broodstock, hatchery and grow out ponds) might be exacerbated by limited biosecurity measures applied to the translocation of fish or their reproductive material. It has been suggested that Acipenserid herpes virus 2 (AciHV2) spread with shipments of live *A. transmontanus* fry from the USA to Italy (Kurobe et al. 2008). Each Australian state and territory has translocation protocols for aquatic animals (see Appendix A [Australia's regulatory control system for finfish health in Australia](#)), but they may not currently include consideration of sturgeon, as sturgeon are not present in Australia. Moreover, the majority of states and territories currently list sturgeon as 'noxious', 'controlled', 'prohibited matter' or 'exotic' under their respective legislation which means they cannot be possessed, reared, handled, transported, bought, sold or dealt with without an exemption or permit, if at all (see section 1.3.3 [Domestic Arrangements](#)). Spread of hazards will also be dependent on the structured surveillance and disease control policies in states or territories. Australian aquaculture operations under state and territory legislation may have disease control programs and report significant disease events but not in all instances (see Appendix A [Australia's regulatory control system for finfish health in Australia](#)). Some of the hazards, such as spring viraemia of carp virus, are notifiable diseases in states and territories and must be reported. Although, state and territory governments would be expected to report on the presence of an unlisted disease agent that has never been reported in Australia. However, reporting is reliant upon the farms identifying and notifying jurisdictions of a possible disease (both endemic and exotic) event. For commercial reasons, overtly diseased fish are unlikely to be transferred between farms. However, for hazards that may be carried by fish or their reproductive material without causing clinical signs, the likelihood of such carrier fish or infected reproductive material being transferred is considered to be high.

Farmed sturgeon may be polycultured with other fish species of commercial or conservation value (LaPatra et al. 1995; Mihailov et al. 2020; Patriche et al. 2002; Rupp et al. 2019). If the polycultured fish become infected or carriers from the imported live sturgeon and were introduced into natural waters, such as to replenish depleted populations, this is a possible pathway for hazards to spread to wild susceptible species.

Spread from wild susceptible species to farmed susceptible species is a potential pathway for establishment and spread. For example, the transfer of infected wild broodstock to hatcheries has been suggested as the cause of establishment of white sturgeon iridovirus (WSIV) in *A. transmontanus* farms in the USA (Drennan et al. 2006; Georgiadis et al. 2001; Hedrick et al. 1992). Aquaculture facilities that rely on the intake of water from the natural environment with limited biosecurity protocols would be susceptible to an outbreak of disease from exposure to infected wild susceptible species or exposure to free hazard in the water. Farmed susceptible species in closed systems, such as secure RAS, or hatcheries with good biosecurity protocols and limited contact to environmental water have a lower likelihood of hazard spread from wild susceptible species.

The replication of disease agents is correlated with water temperature, which is reflected as temperature ranges for infection and clinical diseases. Increasing water temperatures due to climate change will shift the balance in favour of either the host or disease agent, changing the establishment and spread of disease. Several fish diseases will likely become more prevalent and difficult to control as water temperatures increase, such as enteric red mouth, furunculosis and koi herpesvirus disease. The risk of infection with viral haemorrhagic septicaemia (VHSV), spring viraemia of carp virus (SVCV) and IHNV may decline as infection generally only establishes when water temperatures are less than 14°C for VHSV and IHNV and 17°C for SCVC. Consequently, the risk of establishment and spread of disease agents will need to be periodically reviewed to account for the changing effect of climate change (Marcos-Lopez et al. 2010).

#### *Susceptibility of Australian species to infection*

Some of the hazards in this BIRA are host-specific and infect only fish species from the same family. For example, sNCLDV only infects fish in the family *Acipenseridae* (Mugetti et al. 2020b). Other hazards have a much wider host range and can infect multiple families and even different classes. For example, frog virus 3 can infect fish, amphibians and reptiles (Chinchar, Waltzek & Subramaniam 2017; OIE 2021d). Hazards that have a wide host range have a higher likelihood of establishing and spreading in Australia.

Australian fish populations are likely to be at least as susceptible to infection with a hazard as the same species found in other regions. In some cases, the Australian populations may be more susceptible as they will not have prior exposure or host adaptation to the hazards. On the other hand, environmental and husbandry conditions that might favour the expression of disease in fish populations in other regions may not be present in Australia. For example, water temperature is an important factor in infection with some hazards such as viral haemorrhagic septicaemia virus where disease typically occurs between 1–12°C ((Jorgensen 1973a) cited in (Meyers & Winton 1995))(Jorgensen 1982b; Smail 1999). Season, or time of year, can affect the likelihood of establishment and spread. For example, the prevalence of IHNV in spring-run, fall-run and summer-run *Oncorhynchus tshawytscha* in the Columbia River Basin, USA from 2000–2012 was on average 27%, 25% and 13% (Hernandez et al. 2021).

Sturgeon are not native to Australia and there are no sturgeon species known to be present in the wild in Australia. The establishment of a wild sturgeon population would depend on many factors including the number of escaped fish from the aquaculture facilities, survival to maturity, physiological tolerance to water temperatures in the local environment and availability of spawning habitats. During the assessment of the potential risks of culturing non-native sturgeon species in Florida, it was considered that the low reproductive rate and specialised spawning habitats greatly decrease the risk of proliferation of escaped non-native sturgeon species (Metcalf & Zajicek 2000).

#### **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 4. It is assumed that the unrestricted import of sturgeon reproductive material will ultimately result in the same PLES as imported live sturgeon because the resulting progeny will be released from the hatchery.

### **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. Impacts on human life and health are the responsibility of the Australian Government Department of Health and Food Standards Australia New Zealand (FSANZ). The department consults with these agencies on assessments for zoonotic agents.

Direct impacts are those on:

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

Indirect impacts are those on:

- new or modified eradication, control, monitoring or surveillance 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.

Considerations for the direct and indirect impacts are summarised in Table 5.

**Table 5 Considerations for each of the direct and indirect impact criterion**

Effects	Criteria	Considerations
Direct	Animal health (production losses in aquaculture and commercial fisheries)	<ul style="list-style-type: none"> <li>The biological effect of disease is typically evaluated in terms of morbidity and mortality.</li> <li>Diseases that reduce the efficiency of production without causing large increases in mortality are more likely to have a significant impact in farmed species than in the natural environment.</li> <li>Data collected in studies of population density, age/size structure and catch rates can show population fluctuations above 'baseline' mortality. However, only major epidemics involving significant mortalities or grossly visible clinical signs are likely to be detected in wild populations.</li> <li>The consequences of establishment of an exotic disease in Australian fish aquaculture and commercial fisheries is assessed in relation to characteristics of the local industry.</li> <li>The burden of impacts of an outbreak of an exotic fish 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.</li> <li>There is no information on the anticipated production or value of the proposed Australian sturgeon aquaculture industry although at least AU\$30 million is intended to be invested (Stakeholder-personal communication).</li> <li>Caviar prices can vary greatly depending on the species of sturgeon, the country of origin and grading of the product. In 2023, <i>Acipenser gueldenstaedtii</i> (Russian sturgeon) caviar from China sells for up to US\$350/kg, <i>Acipenser schrenckii</i> (Japanese sturgeon) caviar from Poland for up to US\$180/kg, <i>Acipenser transmontanus</i> (white sturgeon) and <i>Acipenser baerii</i> (Siberian sturgeon) caviar from Italy for up to US\$10,942/kg (Alibaba 2023).</li> <li>World farmgate sturgeon meat prices can vary. In 2022, the price for <i>Acipenseridae</i> meat fillets in France was €15 (US\$15.79)/kg and €7.50 (US\$7.89)/kg for gutted sturgeon (FAO 2022b). In 2023, <i>Acipenser fulvescens</i> (lake sturgeon) meat fillets from Canada sell for US\$4.63/kg (Alibaba 2023).</li> <li>While the most common viral diseases of sturgeon are only known to infect sturgeon species, sturgeon can carry and transmit other disease agents that could affect other domestic aquaculture and wild-caught fish industries.</li> <li>In Australia, the main aquaculture finfish species are salmonids, Southern Bluefin tuna and barramundi with several other fish species cultured in lower volumes. The Australian salmonid aquaculture industry produced 84,341 tonnes of product in 2020–21 with an estimated value of AU\$1 billion (Tuynman &amp; Dylewski 2022).</li> <li>The main target species for wild fisheries include tuna, sardines, coral trout and flathead with an extensive list of other finfish species also wild-caught in Australia (Patterson et al. 2021; Tuynman &amp; Dylewski 2022).</li> <li>This BIRA assumes that farmed and wild susceptible species (including native species) in Australia would be at least as susceptible to infection as reported under similar conditions in other countries.</li> </ul>
	The environment (native animals/plants, and non-living environment)	<ul style="list-style-type: none"> <li>The establishment of a new disease could affect the survival of native species not farmed or otherwise commercially exploited.</li> <li>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.</li> </ul>

## Import of live sturgeon for aquaculture

Effects	Criteria	Considerations
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	<ul style="list-style-type: none"> <li>• Australia has a highly developed animal health system that can thoroughly investigate disease problems.</li> <li>• 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.</li> <li>• The costs associated with controlling and monitoring a fish disease outbreak would be substantial for the Australian, state and territory governments and to the aquaculture and fishery industries.</li> <li>• A conservative approach was taken in this BIRA, considering the high cost and time associated with attempts to eradicate new aquatic animal diseases and the low probability of success.</li> </ul>
	Economic (domestic trade effects and impact on other associated industries)	<ul style="list-style-type: none"> <li>• 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 including processors and retailers, may also suffer significant production losses.</li> <li>• Indirect impacts would also likely affect farms that are free of infection and would be most felt in those parts of Australia where aquaculture and wild catch fishing makes a significant contribution to the overall local economy.</li> <li>• Public perception can significantly affect the markets for products intended for human consumption. For example, infection with <i>A. salmonicida</i> can cause visible lesions on the infected fish. Affected product would be unacceptable to the consumer for reasons of quality and aesthetic appeal.</li> <li>• Domestic trade and movement restrictions may apply to wild susceptible species fished from areas impacted by an outbreak.</li> <li>• It is not easy to quantify 'production' in the context of recreational fisheries. Although loss of spending by recreational fishers is likely to have economic impacts to rural and regional areas.</li> </ul>
	Economic (international trade effects)	<ul style="list-style-type: none"> <li>• In 2020–21, Australia exported AU\$509 million of finfish, shark and ray products including AU\$144 million of tuna and AU\$259 million of salmonids (Tuynman &amp; Dylewski 2022).</li> <li>• Several countries have implemented strong import requirements or prohibited the importation of live, fresh and frozen fisheries products to prevent disease incursions.</li> <li>• Some hazards could have an immediate impact on export market access for a range of commodities, even if only reported from a single aquaculture facility.</li> <li>• If an exotic disease were to become established, Australia could use zoning to maintain access to international markets for live fish and, if required, non-viable product, noting that importing countries may not necessarily accept zoning arrangements.</li> </ul>

## Import of live sturgeon for aquaculture

Effects	Criteria	Considerations
	Environment (biodiversity, endangered species and the integrity of ecosystems)	<ul style="list-style-type: none"> <li>• The potential loss of biodiversity if a hazard were to be introduced, establish and spread, would be of concern to the Australian community.</li> <li>• Australia has one of the most diverse fish and frog faunas in the world (ABARES 2015; Tyler 1997).</li> <li>• 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 (see <a href="#">EPBC Act List of Threatened Fauna</a>).</li> <li>• In drawing conclusions on the likely impact of exotic disease on the environment, the department considered overseas data on the species that are susceptible, the effect of infection and the influence of the physical environment on the outcome of infection.</li> </ul>
	Social (changes in tourism, side effects from control measures, and loss of social amenity)	<ul style="list-style-type: none"> <li>• In the event of a disease outbreak, communities where aquaculture production is a significant employer and/or plays a major role in the local community, are expected to experience the most significant social impacts.</li> <li>• Loss of social amenity by recreational fishers due to implementation of a movement restricted area or reduction in fishing interest could occur (McManus et al. 2011).</li> <li>• Loss of culturally significant species will also have social impacts if a disease outbreak were to affect important species for indigenous cultural fishing.</li> </ul>

## 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 geographical 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 states and territories).

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, irreversible and likely to disturb either economic viability or the intrinsic value of the criterion.

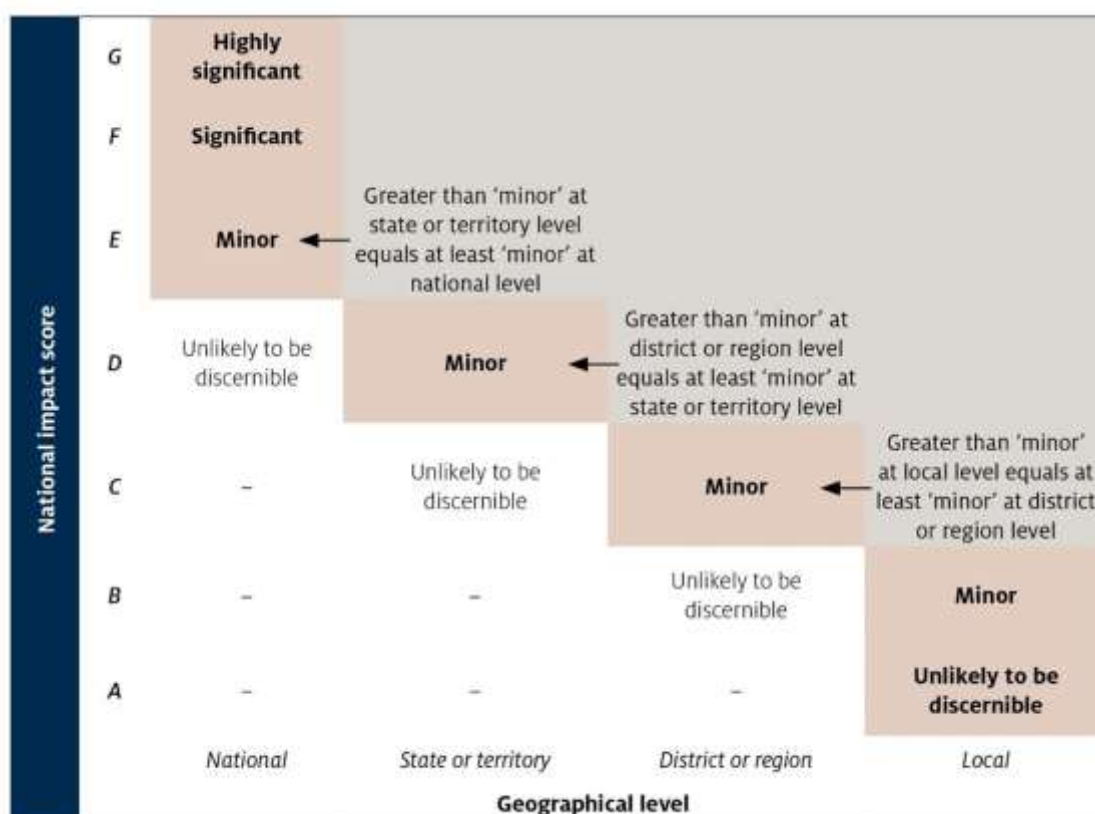
In assessing direct and indirect impacts, impacts were not considered more than once and the frame of reference was the impact of each hazard on the Australian community, rather than on the directly affected parties. A related consideration is the persistence of an effect. If the effect is prolonged, as would be the case if the hazard were expected to persist for several production cycles, or if restocking following eradication programs was expected to take several generations, the consequences were considered greater. If an effect is not expected to be prolonged, then consequences are considered to be less significant. When assessing the impacts under the outbreak scenario, consideration was also given on the potential impact of the establishment of the hazard on an established sturgeon industry in Australia, and not only a developing industry.

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 the 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 in a local area, 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



Rule	Impact scores for each direct and indirect criteria	Overall impact
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

#### Determination of likely consequences for the outbreak scenario

The 'likely consequences' for the outbreak scenario for each exposure group were determined by combining the 'likelihood of establishment and spread' (see section 4.2.5 [Estimation of partial likelihood of establishment and spread](#)) with the 'overall impact' (see [Step 2: Combining direct and indirect impacts](#)) using the matrix shown in Figure 6.

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

#### 4.2.6 Risk estimation

'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 two exposure groups
- combining the two partial annual risks to give an estimate of 'overall annual risk'.

#### 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 4.2.4 [Determination of the partial annual likelihood of entry and exposure](#)) with the estimate of 'likely consequences' (see section 4.2.5 [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

#### Estimation of overall annual risk

The overall annual risk is obtained by combining the PAR (see section 4.2.6 [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.

**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

The result of this process was an estimate of the overall annual risk of introducing a hazard through importation of live sturgeon or their reproductive material for aquaculture purposes. This is the final output of the unrestricted risk assessment.

## 4.3 Risk management

The WOA Code (chapter 2.1) divides risk management into four components:

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

### 4.3.1 Risk evaluation

Risk evaluation is the process of comparing the unrestricted risk estimated in the risk assessment with the WOA member's appropriate level of protection (ALOP).

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

The process for using a benchmark for evaluating risks is:

- For each hazard, the level of risk associated with the unrestricted importation of live sturgeon or their reproductive material is estimated using the process described in this chapter.
- The unrestricted risk (overall annual risk) is then evaluated to determine where it falls in relation to Australia's ALOP.
  - If the unrestricted risk is 'negligible' or 'very low', then it achieves Australia's ALOP and biosecurity measures are not required for that hazard.
  - If the unrestricted risk is 'low', 'moderate', 'high' or 'extreme', then biosecurity measures are identified and the risk is recalculated (referred to as 'restricted risk') with the biosecurity measure(s) applied.
- Where the subsequently restricted risk is 'very low' or 'negligible', that biosecurity measure(s) is considered acceptable for that hazard.

### 4.3.2 Option evaluation

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

Risk management options may be imposed pre-border with the purpose of reducing the likelihood of hazards entering Australia, or post-arrival aiming to prevent the exposure and/or establishment and spread of the hazard in susceptible local populations. In this BIRA, detailed consideration of numerous biosecurity measures for imported live sturgeon or their reproductive material was

undertaken and documented (see chapter [5 Potential biosecurity measures for the importation of live sturgeon or their reproductive material](#)).

#### **Analysis of risk management options**

As a member of the World Trade Organization, and as a partner in free trade agreements, Australia has obligations to allow trade where the science says it is safe to do so. When considering risk management options, the department considers whether the biosecurity measure achieves Australia's ALOP and if it is operationally feasible. Any biosecurity measure which the department considers practical and effective at managing risk must be offered as an option for importers and exporters. It becomes a decision for importers, and ultimately the consumer, as to whether the cost for them to import a product is financially and commercially viable. This is not a decision for the department to make.

### **4.3.3 Implementation**

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

### **4.3.4 Monitoring and review**

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

The department is responsible for monitoring and reviewing any applied biosecurity measures to enable the safe importation of live sturgeon or their reproductive material.

## **4.4 Risk communication**

Risk communication is defined in the WOA 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' (OIE 2021a).

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 BIRA enables stakeholder feedback on draft conclusions and recommendations about Australia's biosecurity policies.

## 5 Potential biosecurity measures for the importation of live sturgeon or their reproductive material

The unrestricted import of live sturgeon or their reproductive material for aquaculture purposes may not achieve Australia's appropriate level of protection (ALOP). If Australia's ALOP is not achieved, then a range of biosecurity measures can be assessed to determine whether they reduce risk to a level that achieves Australia's ALOP. Biosecurity measures are aimed at reducing the likelihood that the import of live sturgeon or their reproductive material 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
- reducing the likelihood that susceptible animals in Australia would be exposed to the hazards.

The least trade restrictive biosecurity measures that could be applied to achieve Australia's ALOP are evaluated. The department can implement these biosecurity measures pre-export and/or post-arrival while the live sturgeon or their reproductive material remain under biosecurity control. After release from biosecurity control, the department has limited powers to require management of biosecurity risks. However, once animals have been cleared for import at the Australian border by the department, state and territory legislation relating to fisheries resource management or animal health may be used by the jurisdictions to manage animals and their products. Alternative biosecurity measures that are demonstrated, to the satisfaction of Australian government, to provide equivalent biosecurity would also be considered.

[Appendix B](#) and [Appendix C](#) provides the risk assessment values for the biosecurity measures found to reduce the overall risk of each hazard to at least *very low* for live sturgeon and their reproductive material, thereby achieving Australia's ALOP.

### 5.1 Sourcing from disease-free stocks

Importing live sturgeon or their reproductive material from a country, zone or compartment that is free from hazards will reduce the likelihood of entry. The department would evaluate a country, zone or compartment for freedom from a hazard to a standard consistent with that recommended by the World Organisation for Animal Health (WOAH) or equivalent. To be satisfied that a country, zone or compartment is free of a given hazard, the department must have formally recognised the competent authority (CA) of that country and be satisfied that the CA has the capacity for disease control, monitoring and surveillance as appropriate for the hazard.

The department would consider applications for self-declaration of historical freedom from a hazard in a country or zone if the hazard has not occurred for at least 10 years.

If countries, zones or compartments have achieved eradication (or the hazard has ceased to occur) or the status of a hazard is unknown, then there must be no clinical, epidemiological or laboratory

evidence that the hazard has occurred in any species during the previous 2 years based on an active targeted surveillance program. The active targeted surveillance program must include a minimum of 2 rounds of sampling per year for at least the last 2 years, and testing for the specified hazards. In some cases, the hazard may be compulsorily notifiable to the CA. The WOAHA *Aquatic animal health code* (WOAH Code) chapter 1.4 '*Aquatic Animal Health Surveillance*', chapter 4.1 '*Biosecurity for aquaculture establishments*', chapter 4.2 '*Zoning and compartmentalisation*', chapter 4.3 '*Application of compartmentalisation*' and the relevant provisions in each disease chapter of the WOAHA Code should be followed as a guide (OIE 2021a).

A rigorous assessment of any application for approval of sourcing from disease-free stocks would be undertaken by the department to ensure that effective biosecurity measures are implemented and maintained throughout the complete supply chain (from source population to point of export). A detailed submission would need to be provided to the department by the CA of the exporting country.

The department would consider on a case-by-case basis exemptions of freedom from a hazard based on the sturgeon species to be imported. However, unequivocal evidence would need to be provided that the sturgeon species are not susceptible to the hazard (e.g. challenge experiments).

Of note, it was stated in 2007 that "leading experts on sturgeon diseases have expressed serious concern regarding the disease status of exotic sturgeons being imported into the U.S. for culture, citing the difficulty and general lack of present capacity to guarantee that such species are free from disease. Specifically, experts have suggested that based on their experience, it is unlikely that foreign origin sturgeon had been examined for the presence of viral agents, and that health certificates or other claims of freedom from viral agents for imported sturgeon are therefore of little value" ((Hedrick 1999) cited in (Tzankova 2007)). It is unknown if there have been improvements since then in guaranteeing sturgeon as disease-free, therefore the risk of spreading disease agents with live animal movements remains.

## **5.2 Sourcing from a premises under the supervision of the competent authority**

Requiring that imported live sturgeon or their reproductive material are sourced from premises under the supervision of the CA may reduce the likelihood of entry of some hazards. The premises would be required to have a documented health monitoring program that includes records of mortalities, reporting of unusual mortalities and ongoing disease surveillance. Supervision of the premises by the CA allow it to attest to the sturgeon's health status and the general health status of the premises. This biosecurity measure makes it more likely that only healthy sturgeon are imported into Australia. However, clinically and subclinically infected sturgeon, sturgeon with parasite infestations or infected or contaminated reproductive material may still be imported. Because of this, sourcing from a premises under the supervision of the CA is unlikely to reduce the entry likelihood of the hazards significantly; however, it is recommended as a general biosecurity measure for both live sturgeon and reproductive material.

### **5.3 Sourcing from a premises that cultures sturgeon only**

Requiring that imported live sturgeon or their reproductive material are sourced from premises that only culture sturgeon species may reduce the likelihood of entry of some hazards. Other fish species farmed for human consumption, such as salmonids or carp, are highly susceptible to several of the hazards and may transmit infections to sturgeon if they are polycultured or share the same water, equipment or other fomite. Indeed, there are several reports of sturgeon becoming infected from these exact scenarios (Kempton et al. 2009; Mohler 2003; Vicenova et al. 2011). Similarly, the culture of amphibians, particularly ranaculture, could be a source of ranaviruses for sturgeon in the same facility. Eliminating other fish and amphibians as a source of infection will assist with only healthy sturgeon being imported into Australia and is therefore also recommended as a general biosecurity measure for both live sturgeon and reproductive material.

### **5.4 Pre-export quarantine**

Restricting imports to live sturgeon that have undergone a pre-export quarantine (PEQ) period may reduce the likelihood of entry of some hazards. Holding sturgeon 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 sturgeon to be monitored for a specified time to ensure they are free of visible signs of disease and parasites prior to export. Specific information on hazard incubation periods in infected sturgeon is limited. Information is available for hazard infections in other fish species and could be used to determine a PEQ period for live sturgeon. However, even with PEQ applied, subclinically infected sturgeon or sturgeon 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 live sturgeon.

### **5.5 Parasite treatment**

Parasite treatment of live sturgeon pre-export will reduce the entry likelihood of those hazards that are ectoparasites. The department may specify a treatment regimen to ensure that the treatments are applied at an appropriate dose, duration and time prior to export. The parasite treatment should be performed in a premises under the supervision of the CA so treatments can be attested to. There is a possibility the treatment regimen will not be sufficient to remove all the ectoparasites on the live sturgeon or the parasiticide will not be effective against all the life stages so reinfestation may occur before or after arrival in Australia. Therefore, the department may also specify that a parasite treatment is applied to live sturgeon post-arrival, and while under biosecurity control.

### **5.6 Batch testing for hazards**

Batch testing for hazards in live sturgeon will reduce the likelihood of entry of some hazards. A batch (epidemiological unit) is defined by the WOA Code as 'a discrete population comprising a group of fish of a single species that share the same potential risk of exposure to a pathogen because they share a common aquatic environment or because management practices make it likely that a pathogen in one group of animals would quickly spread to other animals' (WOAH 2022a). Only those batches that test negative for the hazard would be permitted import. Batch testing may occur either pre-export or post-arrival in Australia using a method and sampling regime approved by the department. In general, the sampling regime should provide at least 95% confidence of detecting the

hazard if it is present at a prevalence of 2% or greater. However, these testing parameters would be determined for any hazard requiring batch testing (noting that a 95% confidence level of detecting a 2% prevalence are considered appropriate when reliable information on the expected prevalence in an infected population is not available) (OIE 2021a).

The effect of batch testing on the magnitude of the reduction in entry likelihood is dependent on a range of factors including the integrity of the sampling regime (including security of the batches), the adherence to the sampling procedures (including appropriate random selection of samples), the availability of effective testing methods (including known diagnostic sensitivity and specificity) and the prevalence of the hazard in the batch of sturgeon. Because of these factors, pre-export batch testing in the exporting country and post-arrival batch testing in Australia will be considered separate biosecurity measures. Of note, pre-export and on-arrival batch testing is recommended in the WOA Code for the importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with the WOA-listed fish diseases when the intention is to establish a new stock for aquaculture (WOAH 2022b).

## **5.7 Egg disinfection**

The disinfection of sturgeon eggs to remove disease agents residing on the surface may reduce the likelihood of entry of some hazards. For example, egg disinfection with iodophor is a common practice to control infectious haematopoietic necrosis virus (IHNV) infections (Bovo et al. 2005a; Goldes & Mead 1995). However, for other hazards, egg disinfection may have no effect, or the effect is unknown (e.g. white sturgeon iridovirus) (Drennan et al. 2006). Further, egg disinfection is not effective when the disease agent is vertically transmitted. Eggs will typically undergo a disinfection procedure as part of standard hatchery protocols, and therefore this option is recommended as a general biosecurity measure for sturgeon eggs.

## **5.8 Post-arrival quarantine**

Requiring imported live sturgeon or their reproductive material to undergo a post-arrival quarantine (PAQ) period in Australia may reduce the entry likelihood of some hazards. Sub-clinically infected live sturgeon are unlikely to be detected prior to export, even after a PEQ period, and could be imported to Australia. If a parasite treatment is not performed on the live sturgeon or does not remove all the ectoparasites leading to reinfestation during transport, infested sturgeon may be imported to Australia. The stress of the transport process may cause sub-clinically infected sturgeon or survivors of a previous infection to succumb to clinical infection on arrival in Australia. Lastly, live sturgeon may be imported in water that contains disease agents that infect or infest the sturgeon during or after transport.

On arrival, the imported live sturgeon would be placed under biosecurity control, inspected for signs of clinical infection or parasites and moved to an approved arrangement (AA) to undergo PAQ. During the PAQ period, the live sturgeon would be observed for the appearance of clinical signs of disease or the presence of parasites. Specific information on hazard incubation periods in infected sturgeon is limited. Information is available for hazard infections in other fish species and could be used to determine the PAQ period for live sturgeon. The PAQ period would also be the appropriate time for parasite treatment to be applied to the live sturgeon, and any samples to be taken for batch testing for hazards as all procedures would be performed under the supervision of the department.



At the end of the PAQ period, only live sturgeon that show no signs of clinical disease or parasites and if required, test negative for specified hazards, would be released from biosecurity control.

The option to hold the live sturgeon in PAQ until they produce a first generation (F1) population was considered. In this scenario, the F1 population would then be cultured for a duration that is sufficient for, and under conditions that are conducive to, the clinical expression of infection with the hazards. Only F1 sturgeon that show no signs of clinical disease and test negative for the specified hazards would be released from biosecurity control. This biosecurity measure of only releasing a F1 population that has tested negative for specific disease agents is recommended in the WOAHA Code for the importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with the WOAHA-listed fish diseases (WOAHA 2022b). However, this option was not considered practical or feasible for imported larvae or juvenile sturgeon since it can take at least 3–4 years under optimal conditions for *Acipenser baerii* to reproduce (Jahrl 2013; UNODC 2016) and 9–12 years for *Huso huso* (Chebanov & Galich 2013). This option may be considered further on a case-by-case basis if sexually mature sturgeon were imported for spawning in Australia.

In the case of imported sturgeon reproductive material, it typically will not show any clinical signs of infection. However, the consignment would still be inspected after being placed under biosecurity control on arrival, followed by transfer to an AA for PAQ. During the PAQ period, the reproductive material would be used to produce sturgeon progeny. This could be done by either fertilising the imported eggs using imported or domestic milt, using the imported milt to fertilise domestic eggs or hatching the imported fertilised eggs. The sturgeon progeny would be grown out to an appropriate size for species identification, observed for the appearance of clinical signs of disease and, if required, batch tested for specified hazards. Only sturgeon progeny that show no signs of clinical disease, test negative for hazards, and complete their PAQ period would be released from biosecurity control.

## 6 Hazard identification

Table 8 shows the list of potential hazards identified as being relevant to the scope of this BIRA and summarises the results of the hazard identification process, including the reason for retaining or not retaining each disease agent.

Many disease agents that infect sturgeon are ubiquitous and may already be present in Australia. There are others that are opportunistic, not reported to be pathogenic, or of uncertain relevance in sturgeon due to limited or insufficient information. All these disease agents 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 4.1 [Hazard identification](#).

The disease agents identified as hazards and retained for risk assessment are listed at the end of this chapter (see section 6.1 [Hazards retained for risk assessment](#)).

Table 8 Hazard identification

Disease agent (disease)	Susceptible species	WOAH- listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<b>Viruses</b>						
Cyprinid herpesvirus 3 ( <i>koi herpesvirus</i> ) (Koi herpesvirus disease)	Primarily a disease of cyprinids but can be detected in other species of fish, including sturgeon	Yes	Yes	No	Yes: WOAHL-listed, not present in Australia, has been detected in sturgeon and can cause significant mortalities in cultured susceptible fish.	(Haenen et al. 2004; Kempter et al. 2009)
Frog virus 3	Amphibians, reptiles, and fish, including sturgeon	Yes	Yes	No – other <i>Ranavirus</i> species are present in Australia including Bohle iridovirus and epizootic haematopoietic necrosis virus	Yes: WOAHL-listed, not present in Australia and may cause mortalities in susceptible species.	(Duffus et al. 2015; Waltzek et al. 2014)
Infectious haematopoietic necrosis virus	Primarily a disease of salmonids but can infect other species of fish, including sturgeon	Yes	Yes	No	Yes: WOAHL-listed, evidence of susceptibility in sturgeon, not present in Australia, and may cause significant mortalities in cultured susceptible fish.	(Bootland & Leong 1999; LaPatra et al. 1995)
Nervous necrosis viruses (with the exception of Red-spotted grouper nervous necrosis virus) (Viral encephalopathy and retinopathy)	Wide range of fish	No	Yes	No	Yes: not present in Australia, viral encephalopathy and retinopathy is included on <i>Australia's National list of reportable diseases of aquatic animals</i> and can cause significant mortalities in cultured susceptible fish.	(Bandín & Souto 2020; Munday, Kwang & Moody 2002)

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Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
Red-spotted grouper nervous necrosis virus (Viral encephalopathy and retinopathy)	Wide range of fish, and has been reported in sturgeon and seahorses	No	Yes	Yes	No: present in Australia and although viral encephalopathy and retinopathy is included on <i>Australia's National list of reportable diseases of aquatic animals it is not subject to control or eradication.</i>	(Athanassopoulou, Billinis & Prapas 2004; WOA 2019)
Spring viraemia of carp virus	Primarily a disease of cyprinids but can infect other species of fish, including sturgeon	Yes	Yes	No	Yes: WOA-listed, not present in Australia, evidence of susceptibility in sturgeon and can cause significant mortalities in cultured susceptible fish.	(Ahne et al. 2002; Vicens et al. 2011)
Sturgeon herpesviruses	Sturgeon	No	Yes	No	Yes: evidence of susceptibility in sturgeon, is not present in Australia, and may cause mortalities in cultured susceptible fish .	(Goodwin 2012; Johnston et al. 2022; Kelley et al. 2005; Kurobe et al. 2008; LaPatra et al. 2014; Mugetti et al. 2020a; Shchelkunov et al. 2009; Walker et al. 2022; Waltzek et al. 2009)
Sturgeon nucleocytoplasmic large DNA viruses (Acipenser iridovirus-European, British Columbia white sturgeon virus, Missouri River sturgeon iridovirus, Namao virus, shortnose sturgeon virus, White sturgeon iridovirus)	Sturgeon	No	Yes	No	Yes: evidence of susceptibility in sturgeon, is not present in Australia, and may cause significant mortalities in cultured susceptible fish.	(Bigarré et al. 2017; Hedrick et al. 1992; Hofsoe-Oppermann et al. 2019; LaPatra et al. 1994; Mugetti et al. 2020a; Mugetti et al. 2020b; Raverty et al. 2003; Rud et al. 2020)

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Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
Viral haemorrhagic septicaemia virus	Primarily a disease of salmonids but can infect other species of fish and sturgeon cell lines	Yes	Yes	No	Yes: WOAH-listed, not present in Australia, may cause significant mortalities in cultured susceptible fish and sturgeon cell lines can be infected with VHSV and display a cytopathic effect.	(Bruch et al. 2016; Ryu et al. 2018)
White sturgeon adenovirus	<i>Sturgeon</i>	No	No	No	No: since the initial report there has been no evidence of adverse consequences associated with the disease agent reported.	(Hedrick et al. 1985)
<b>Bacteria</b>						
<i>Acinetobacter</i> species	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Kayis et al. 2017)
<i>Aeromonas hydrophila</i>	Wide range of fish, including sturgeon and prawns	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(AQIS 1999a; Kayis et al. 2017; Noga 2000)

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Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Aeromonas salmonicida</i> (typical strain)	Primarily a disease of salmonids but can infect other species of farmed fish, including farmed sturgeon	No	Yes	No – some atypical strains have been reported in Australia	Yes: wide host range, including sturgeon, the typical strain is not present in Australia, and may cause mass mortalities in cultured susceptible fish.	(Dallaire-Dufresne et al. 2014; Mohler 2003).
<i>Aeromonas sobria</i>	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Kayis et al. 2017)
<i>Aeromonas veronii</i>	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Ma et al. 2009; Sinclair et al. 2016)
<i>Bacillus mycoides</i>	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Kayis et al. 2017)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Carnobacterium</i> species	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Ciulli et al. 2020; Kim et al. 2015)
<i>Chlamydia</i> species (Epitheliocystis)	Wide range of fish, some species specific to sturgeon	No	No	Yes	No: <i>Chlamydia</i> spp. are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication, and no evidence of adverse consequences associated with the sturgeon specific species.	(Groff et al. 1996)
<i>Chryseobacterium joostei</i>	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(Gholamhosseini et al. 2018)
<i>Citrobacter freundii</i>	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Ciulli et al. 2020)

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Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Clostridium perfringens</i>	Wide range of fish, including sturgeon, and other animal species, including humans	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication. <i>C. perfringens</i> is widespread in the environment and in the gut of people and animals. Can cause gastro illness in humans.	(Brocca et al. 2022)
<i>Edwardsiella tarda</i>	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(AQIS 1999a; Yang et al. 2018)
<i>Flavobacterium</i> species	Wide range of fish, including sturgeon	No	Yes	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(Bauer, Pugachev & Voronin 2002; Karatas et al. 2010; Pelkola et al. 2012)
<i>Flexibacter</i> species	Wide range of fish, including sturgeon and crustaceans	No	No	Yes	No: present in Australia, is not included <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Handler, Soltani & Percival 1997)



# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Mycobacterium chelonae</i>	Sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of significant adverse consequences associated with the disease agent reported.	(Antuofermo et al. 2014; Ashburner 1977; Humphrey et al. 1987; Li et al. 2014; Strike et al. 2017)
<i>Mycobacterium ulcerans</i>	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Zhang et al. 2018)
<i>Mycobacterium salmoniphilum</i>	Primarily a disease of salmonids but can infect sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Righetti et al. 2014)
<i>Pasteurella</i> species	Wide range of fish, including sturgeon	No	No	Yes – some species present in Australia	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Costinar et al. 2010)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Pseudomonas</i> species	Sturgeon	No	No	Yes – some species present in Australia	No: <i>Pseudomonas alcaligenes</i> is known to infect cultured <i>Acipenser sinensis</i> (Chinese sturgeon), but <i>P. alcaligenes</i> is present in Australia and no evidence of adverse consequences associated with the disease agent reported.	(Twigg & Socha 2001; Xu et al. 2015)
<i>Staphylococcus saprophyticus</i>	Sturgeon and other aquatic and terrestrial animals	No	Yes	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(Wu et al. 2023; Zhou 2021)
<i>Staphylococcus sciuri</i>	Sturgeon and other aquatic and terrestrial animals	No	Yes	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(Zhang et al. 2022)
<i>Streptococcus iniae</i>	Wide range of fish, including sturgeon	No	Yes	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(Bromage, Thomas & Owens 1999; Deng et al. 2017; Muhammad et al. 2020; Pierezan et al. 2020)
<i>Vibrio alginolyticus</i>	Sturgeon and other aquatic animals including prawns	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Costinar et al. 2010)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Vibrio anguillarum</i>	Wide range of fish, including sturgeon, and other aquatic animals including prawns	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Munday et al. 1992; Zaharia & Dumitrescu 2011)
<i>Vibrio metschnikovii</i>	Sturgeon and other aquatic and terrestrial animals	No	Yes	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(Carson et al. 2020; Xiao et al. 2022)
<i>Vibrio vulnificus</i> (biotype 2)	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Safari et al. 2015)
<i>Yersinia ruckeri</i> – Hagerman strain (Enteric redmouth disease, Yersiniosis)	Wide range of fish, including sturgeon and salmonids	No	Yes	No – other strains of <i>Yersinia ruckeri</i> are present in Australia	Yes: wide host range, not present in Australia, evidence sturgeon are involved in epidemiology and may cause significant mortalities in cultured susceptible fish.	(Barnes 2011; Shaowu et al. 2013; Vuillaume et al. 1987)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<b>Fungi/oomycete</b>						
<i>Achlya</i> species	Wide range of fish, freshwater and marine crustaceans	No	No	Yes – some species reported in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<i>Aphanomyces</i> species	Wide range of fish, including sturgeon	No	No	Yes – some species reported in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<i>Dactyulus</i> species	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<i>Leptomitius lacteus</i>	Wide range of fish, including sturgeon (affects eggs/larvae only)	No	No	No	No: evidence of susceptibility in sturgeon but only infects sturgeon eggs and there is no evidence of significant adverse consequences associated with the disease agent reported in susceptible species.	(Czeczuga, Semeniuk & Czeczuga-Semeniuk 2012)
<i>Pleistophora sulci</i>	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Saprolegnia</i> species (Saprolegniosis)	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the disease agent reported.	(Kayis et al. 2017; Noga 2000)
<i>Veronaea botryosa</i> (systemic phaeohyphomycosis)	Sturgeon and other aquatic and terrestrial animals	No	Yes	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(Soto et al. 2017a)
<i>Zeptologia</i> species	Sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<b>Protozoa</b>						
Amoebozoa (Amoebic Gill Disease)	Wide range of fish, including sturgeon	No	Yes	Yes – some species reported in Australia	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(Hughes, Smith & Luoma 2001)
<i>Apiosoma</i> species	Wide range of fish, including sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
Ciliates (including <i>Ichthyophthirius multifiliis</i> )	Wide range of fish, including sturgeon	No	No	Yes – some species reported in Australia	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the disease agent reported.	(AQIS 1999a)
<i>Cryptobia</i> species	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<i>Eimeria</i> species	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
Euglenozoa - including <i>Enteromyxum</i> species, <i>Henneguya</i> species, <i>Kudoa</i> species, <i>Myxobolus</i> species, <i>Parvicapsula</i> species, <i>Sphaerospora</i> species, <i>Thelohanellus</i> species, <i>Unicapsula</i> species	Wide range of fish, including sturgeon	No	No	Yes – some species reported in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the disease agent reported.	(Noga 2000)
<i>Glugea</i> species	Wide range of fish, including sturgeon	No	No	Yes	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002; Su 1993; Vagelli et al. 2005)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Haemogregarina acipenseris</i>	Wide range of fish, including sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<i>Hexamita truttae</i>	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<i>Nitzschia</i> species	Sturgeon	No	Yes	No	No: only reported in sturgeon during marine life phase and no evidence of adverse consequences associated with the disease agent in freshwater reported.	(Bauer, Pugachev & Voronin 2002)
<i>Trichodina</i> species	Wide range of fish, including sturgeon	No	No	Yes – some species present in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002; Moghaddam et al. 2010)
<i>Trypanosoma anura</i>	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<i>Zschokkella sturionis</i>	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<b>Metazoa</b>						
<i>Argulus alosae</i> , <i>Argulus coregoni</i> , <i>Argulus flavescentis</i> , <i>Argulus foliaceus</i> and <i>Argulus stizostethii</i> (Fish louse)	Wide range of fish, including sturgeon, cyprinids and salmonids	No	Yes	No	Yes: wide host range, not present in Australia, can be vectors for other diseases and may cause mass mortalities in juvenile cultured fish.	(Bauer, Pugachev & Voronin 2002; Munroe et al. 2011; Paperna 1991; Popielarczyk & Kolman 2013)
<i>Caligus elongatus</i>	Wide range of fish, including sturgeon and salmonids	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(AQIS 1999a)
Cestodes - including <i>Amphilina foliacea</i> , <i>Amphilina japonica</i> , <i>Bothriomonus fallax</i> , <i>Cyathocephalus truncates</i> , <i>Eubothrium acipenserinum</i>	Wide range of fish and turtles, including sturgeon	No	No	Yes – some species reported in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the sturgeon species reported.	(Bauer, Pugachev & Voronin 2002; Bosi et al. 2005)
<i>Dermocystidium</i> species	Wide range of fish, including sturgeon	No	No	Yes	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no evidence of adverse consequences associated with the disease agent reported.	(Shamsi et al. 2020; Yanong 2003)



# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
<i>Diclestium oblongum</i>	Sturgeon	No	No	No	No: only reported in sturgeon during marine life phase and no evidence of adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
Digeneans - including <i>Acrolichanus auriculatus</i> and <i>Skrjabinopsolus</i> species	Wide range of fish, including sturgeon	No	No	Yes – some species reported in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the sturgeon species reported.	(Bauer, Pugachev & Voronin 2002)
<i>Ergasilus sieboldi</i>	Wide range of fish, including sturgeon	No	Yes	No	Yes: wide host range, not present in Australia, and may cause severe gill damage, anaemia and secondary infection, sometimes resulting in heavy losses of fish stocks.	(Bauer, Pugachev & Voronin 2002; Liberman & Voropaeva 2018; Popielarczyk & Kolman 2013; Schäperclaus 1992)
<i>Ichthyophonus hoferi</i>	Wide range of fish, including sturgeon	No	No	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the disease agent reported.	(Hershberger et al. 2010c)
<i>Lernaea cyprinacea</i>	Wide range of fish, including sturgeon	No	Yes	Yes	No: present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication.	(AQIS 1999b; Bauer, Pugachev & Voronin 2002)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH-listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
Monogeneans - including <i>Diclybothrium armatum</i> and <i>Diclybothrium hamulatum</i>	Sturgeon	No	No	Yes - some species reported in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the sturgeon species reported.	(Bauer, Pugachev & Voronin 2002)
Nematodes - including <i>Anisakis schupakowi</i> , <i>Cucullanus</i> species, <i>Hysterothylacium</i> species and <i>Piscicapillaria tuberculata</i>	Wide range of fish, including sturgeon	No	No	Yes - some species reported in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the sturgeon species reported.	(Bauer, Pugachev & Voronin 2002; Noga 2000)
<i>Paradydibothrium padficum</i>	Sturgeon	No	No	No	No: only reported in sturgeon during marine life phase and no evidence of adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)
<i>Polypodium hydriforme</i>	Sturgeon	No	Yes	No	Yes: evidence of susceptibility in sturgeon, not present in Australia, and infects eggs, which decreases the quality of the caviar and may reduce the reproductive potential of the host.	(Okamura et al. 2020; Raikova 2002)
<i>Pseudotracheilastes stellatus</i>	Sturgeon	No	No	No	No: no evidence of significant adverse consequences associated with the disease agent reported.	(Bauer, Pugachev & Voronin 2002)

# Import of live sturgeon for aquaculture

Disease agent (disease)	Susceptible species	WOAH- listed disease? (Yes/No)	Adverse consequences in Australia? (Yes/No)	Present in Australia? (Yes/No)	Retained for risk assessment? (Yes/No: reason)	References
Trematodes - including <i>Aspidogaster limacoides</i> , <i>Deropristis hispida</i> , <i>Diplostomum spathaceum</i> , <i>Pristicola</i> species, <i>Rhipidocotyle kovalae</i>	Wide range of fish, including sturgeon	No	No	Yes – some species reported in Australia	No: some species are present in Australia, is not included on <i>Australia's National list of reportable diseases of aquatic animals</i> and is not subject to control or eradication and no significant adverse consequences associated with the sturgeon species reported.	(Bauer, Pugachev & Voronin 2002; Choudhury 2009; Moghaddam et al. 2010)

## 6.1 Hazards retained for risk assessment

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

- *Aeromonas salmonicida salmonicida* (typical strain)
- *Argulus alosae*, *Argulus coregoni*, *Argulus flavescens*, *Argulus foliaceus* and *Argulus stizostethii*
- Cyprinid herpesvirus 3 (koi herpesvirus)
- *Ergasilus sieboldi*
- Frog virus 3
- Infectious haematopoietic necrosis virus
- Nervous necrosis virus (barfin flounder NNV, tiger puffer NNV, striped jack NNV, reassortant NNV)
- *Polypodium hydriforme*
- Spring viraemia of carp virus
- Sturgeon alloherpesviruses
- Sturgeon nucleocytoplasmic large DNA viruses
- Viral haemorrhagic septicaemia virus
- *Yersinia ruckeri* (Hagerman strain only).

# 7 *Aeromonas salmonicida salmonicida* (typical strain)

## 7.1 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* (Dallaire-Dufresne et al. 2014; Menanteau-Ledouble et al. 2016):

- *A. salmonicida achromogenes* – atypical strain
- *A. salmonicida masoucida* – atypical strain
- *A. salmonicida pectinolytica* – atypical strain
- *A. salmonicida salmonicida* – typical strain
- *A. salmonicida smithia* – atypical strain.

*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 2022a) 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 2001; Hiney & Oliver 1999).

Infection with typical *A. salmonicida* is not listed as a disease notifiable to WOA (OIE 2020) but it is on Australia’s *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). 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.

## 7.2 Technical information

### 7.2.1 Agent properties

Typical *A. salmonicida* is a non-motile, gram-negative rod bacterium classified within the family *Aeromonadaceae*, order *Aeromonadales* (Janda & Abbott 2010). 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* 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 (Agriculture 2009)). Survival times in water are dependent on many factors including temperature, salinity, ultraviolet radiation and the presence of organic matter (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 (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 (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 (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 three salmonid species (*Oncorhynchus mykiss*, *O. kisutch* and *Oncorhynchus tshawytscha*) 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 (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 2001; Mainous, Kuhn & Smith 2011).

## 7.2.2 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 2001). Non-salmonid species which are reported to be susceptible to infection (N= natural exposure; E= experimental exposure) with typical *A. salmonicida* include, but are not limited to:

- *Acipenser oxyrinchus*<sup>N</sup> (Atlantic sturgeon) (Mohler 2003)
- *Carassius auratus*<sup>N, E</sup> (goldfish) (Lian et al. 2020)
- *Catostomus commersoni*<sup>N</sup> (white sucker) (Ostland, Hicks & Daly 1987)
- *Centrolabrus exoletus*<sup>N</sup> (rock cook) (Treasurer & Laidler 1994)
- *Coregonus peled*<sup>N</sup> (northern whitefish) (Long et al. 2023)
- *Coreius guichenoti*<sup>N</sup> (largemouth bronze gudgeon) (Long et al. 2016)
- *Ctenolabrus rupestris*<sup>N</sup> (goldsinny) (Treasurer & Laidler 1994)
- *Ctenopharyngodon idella*<sup>E</sup> (grass carp) (Long et al. 2016)
- *Cyclopterus lumpus*<sup>N</sup> (lumpfish) (Chakraborty et al. 2022)
- *Dicentrarchus labrax*<sup>N, E</sup> (European bass) (Fernández-Álvarez et al. 2016)
- *Gadus morhua*<sup>N</sup> (Atlantic cod) (Boily, Malcolm & Johnson 2019)
- *Hippoglossus hippoglossus*<sup>E</sup> (Atlantic halibut) (Bricknell et al. 1999)
- *Labridae* species<sup>N</sup> (Wrasses) (Treasurer & Laidler 1994)
- *Luxilus cornutus*<sup>N</sup> (common shiner) (Ostland, Hicks & Daly 1987)
- *Perca flavescens*<sup>N</sup> (Yellow perch) (Diamanka et al. 2013)
- *Perca fluviatilis*<sup>N</sup> (European perch, redfin) (Skrodenyte-Arbaciauskiene et al. 2010)
- *Petromyzon marinus*<sup>N</sup> (sea lamprey) (Diamanka et al. 2013)
- *Polyodon spathula*<sup>N, E</sup> (Mississippi paddlefish) (Ford, Cipriano & Penniston 1994)
- *Sander lucioperca*<sup>E</sup> (pike-perch) (Schulz et al. 2020)
- *Scophthalmus maximus*<sup>N, E</sup> (turbot) (Farto et al. 2011; Toranzo & Barja 1992)
- *Sparus aurata*<sup>N</sup> (gilthead seabream) (Real et al. 1994).

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). Infected *A. oxyrinchus* were 40 g and *P. spathula* were fingerlings (12 cm) and juveniles (Ford, Cipriano & Penniston 1994; Mohler 2003). There are differences in susceptibility of fish species to furunculosis. For example, *O. mykiss* (rainbow trout) are generally considered to be mostly resistant to infection with typical *A. salmonicida* whereas *S. trutta* (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

### *Sturgeon*

No reports were found on the prevalence of typical *A. salmonicida* in farmed or wild sturgeon.

### *Salmonids*

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 *S. salar* held at a facility in the USA (Cipriano et al. 2001). A survey of wild fish collected from Michigan rivers, USA in 2005–2010 detected typical *A. salmonicida* at a prevalence of 9.6% (n=2115) with 20.5% (n=806) in *Oncorhynchus tshawytscha* (chinook salmon), 6% (n=301) in *S. salar*, 2.8% (n=623) in *O. kisutch* (coho salmon) and 0.8% (n=385) in *O. mykiss* (Diamanka et al. 2013). There were 11 farm-level diagnoses of furunculosis in seawater-reared *S. salar* in British Columbia, Canada between 2002–2016 (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 2013–2017, 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 *P. marinus* collected from Lake Ontario, Canada tested positive for typical *A. salmonicida* (Faisal, Eissa & Elsayed 2007).

## Mortalities

### *Sturgeon*

An outbreak of typical *A. salmonicida* in *P. spathula* fingerlings at a hatchery in Arkansas, USA, in 1992 caused 90% mortality of approximately 7,000 fish (Ford, Cipriano & Penniston 1994). Typical *A. salmonicida* caused mortality (no numbers given) in *A. oxyrinchus* (40 g) in a hatchery also in the USA (Mohler 2003).

### *Salmonids*

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).

### *Other fish*

There are limited recent publications about mortalities in wild or farmed populations due to typical *A. salmonicida*. In 1992, an outbreak in *S. maximus* in net cages in Spain caused 15% cumulative mortality out of approximately 1,200 fish over one month (Toranzo & Barja 1992). *C. rupestris* and



*C. exoletus* polycultured with *S. salar* in net pens in Scotland in 1991–1992 suffered 55% mortality due to typical *A. salmonicida* infection (Treasurer & Laidler 1994). During 2008, mass mortalities (no numbers given) in wild *P. fluviatilis* in North Lithuanian rivers were attributed to typical *A. salmonicida* (Skrodenyte-Arbaciauskiene et al. 2010). Typical *A. salmonicida* infections in *D. labrax* cultured in net pens on the Mediterranean Coast of Spain in 2012 caused cumulative mortalities of 3.8% (Fernández-Álvarez et al. 2016).

### 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 2001; Dallaire-Dufresne et al. 2014). It was transmitted to *A. oxyrinchus* by cohabitation with infected *Salvelinus fontinalis* (brook trout) resulting in clinical disease and mortalities (Mohler 2003). 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 2001; 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 *S. salar* and *O. mykiss* 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 *S. trutta* 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 *O. tshawytscha* 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 2001; 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). *O. mykiss* (mean weight 25 g) survivors of experimental infection with typical *A. salmonicida* shed on average  $3 \times 10^4$ – $10^5$  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 (Agriculture 2009)).

Contaminated equipment has been implicated in the spread 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).

### Infectious dose

The minimum infective dose of typical *A. salmonicida* for *S. salar* (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 *S. salar* (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 *O. mykiss* (25 g) following bath exposure for 12 hours was  $10^8$  CFU/mL and for *S. maximus* (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  $3 \times 10^5$  CFU/mL for *O. mykiss* (25 g) and  $2 \times 10^4$  CFU/mL for *S. maximus* (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 *S. salar* (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 *O. tshawytscha* (1.2 g) by bath exposure (Ogut & Reno 2005). *S. salar* (approximately 600 g) injected with 100  $\mu$ L of typical *A. salmonicida* ( $3.05 \times 10^7$  CFU/mL) induced mortality by 6 dpi (Yi et al. 2016). Cohabitation of healthy *O. tshawytscha* (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 *C. idella* (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 *S. maximus* (11 g) to  $10^6$  CFU/mL typical *A. salmonicida* for 1 hour caused 100% mortality 7 dpi (Farto et al. 2011). The  $LD_{50}$  of typical *A. salmonicida* in *C. auratus* was  $4.5 \times 10^6$  CFU/g following intraperitoneal injection (Lian et al. 2020). *D. labrax* (mean weight 7.5 g), *S. maximus* (mean weight 5.0 g) and *O. mykiss* (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 *H. hippoglossus* (weight 154–254 g) and *S. salar* (weight 93–289 g) with  $10^3$ – $10^8$  CFU/fish showed *H. hippoglossus* was more resistant to infection compared to *S. salar*, with  $1.25 \times 10^6$  and  $\leq 1 \times 10^2$  CFU/fish the minimum lethal doses, respectively (Bricknell et al. 1999).

## 7.2.3 Pathogenesis

### Tissue tropism

Typical *A. salmonicida* gains 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 *S. salar* eggs ranged from  $5.0 \times 10^2$ – $1 \times 10^7$  CFU/g of egg (Cipriano et al. 2001). Dead *O. tshawytscha* 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 *O. keta* and *O. gorbuscha* 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 *S. trutta* (Hiney & Oliver 1999) and  $10^6$  CFU/g were detected in mucus of *S. salar* 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 *S. salar* was  $10^{10}$  CFU/mL (Rose, Ellis & Munro 1989).

## 7.2.4 Diagnosis

### Clinical signs

#### *Sturgeon*

Infected sturgeon displayed lethargy, uncoordinated swimming, difficulty maintaining equilibrium, discolouration with a lighter-than-normal appearance, redness at the base of fins and protrusion of the uro-genital opening along with a bloody discharge. In some instances, discrete furuncles were present on the skin (Mohler 2003).

#### *Salmonids*

In salmonids, infection with typical *A. salmonicida* can result in peracute, acute or chronic 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 2001; 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 2001; Hiney & Oliver 1999). Furuncles involving skin, muscle or the viscera may be present but are usually restricted to the chronic infection (Cipriano & Bullock 2001; Hiney & Oliver 1999). Chronic infection is typically reported in older fish (subadults and adults) that have become more refractory to the disease or in more resistant species (Brocklebank 1998; Cipriano & Bullock 2001; 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 2001; Dallaire-Dufresne et al. 2014; Hiney & Oliver 1999). There can be evidence of healing of the furuncles in chronic infections (Agriculture 2009). In some cases, such as when fish are subclinically infected or have survived an infection, no clinical signs are observed (Cipriano & Bullock 2001; Hiney & Oliver 1999; Menanteau-Ledouble et al. 2016).

#### *Other fish*

Infected eels, such as *Anguilla rostra* and *Anguilla japonica*, 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). *S. maximus* (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 *C. guichenoti*, *C. auratus*, *P. fluviatilis* and *P. marinus* (Faisal, Eissa & Elsayed 2007; Lian et al. 2020; Long et al. 2016; Rupp et al. 2019).

## Pathology

### Sturgeon

No pathological findings have been described for infected sturgeon.

### Salmonids

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 2001; Dallaire-Dufresne et al. 2014; Hiney & Oliver 1999; Menanteau-Ledouble et al. 2016).

### Other fish

Histological examination of infected *S. maximus* 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 *D. labrax* (Fernández-Álvarez et al. 2016). Infected *C. auratus* 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 2001).

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).

## 7.2.5 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). An outbreak in *P. spathula* fingerlings at a hatchery in the USA was treated with terramycin that reduced mortality (Ford, Cipriano & Penniston 1994).

### 7.2.6 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. An experimental study did show that *A. baerii* (Siberian sturgeon) (40 g, 2 months old) exposed to a bacterin vaccine could induce anti-typical *A. salmonicida* antibodies but it is unclear if it is being used commercially (Kolman 2002).

Improved management and husbandry practices have led to decreased mortality rates and outbreaks of clinical disease (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 2001; 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 2001).

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 2001; Cipriano & Heartwell 1986; Gjedrem 2010; Kjoglum et al. 2008; Zhang et al. 2011).

### 7.2.7 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 (Somerset et al. 2005a). Furunculosis was still listed as a major concern for European *O. mykiss* farming in 2020 (EURL for Fish and Crustacean Diseases 2021). In 2019, *S. salar* production in Europe was 1.6 million tonnes and production of *O. mykiss* 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).

### 7.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon or their reproductive material as import is not permitted.

There are biosecurity measures to manage the risk of typical *A. salmonicida* in imported ornamental fish for display purposes and salmonid fish for human consumption (see [Appendix D](#)).

## 7.3 Risk assessment

Based on [chapter 4](#) and the technical information about typical *A. salmonicida* presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of typical *A. salmonicida* meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 7.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that typical *A. salmonicida* is present in all source countries.
- Typical *A. salmonicida* is expected to infect sturgeon that would be of a life stage exported to Australia.
- There is one report of typical *A. salmonicida* in farmed sturgeon causing mortality (no numbers given) in a hatchery in the USA (Mohler 2003). No reports were found on the prevalence of typical *A. salmonicida* in farmed or wild sturgeon.
- The prevalence of typical *A. salmonicida* in sturgeon reproductive material is unknown but there are reports of the bacteria contaminating salmonid eggs (Cipriano et al. 2001).
- The bacterial load of typical *A. salmonicida* in infected imported live sturgeon or reproductive material is likely to be sufficient to cause infection in susceptible species.
- Typical *A. salmonicida* can persist outside its host in marine, brackish and freshwater environments for an extended period.
- Inspection may detect sturgeon showing clinical signs of infection with typical *A. salmonicida* and remove them before export. Sturgeon sub-clinically infected or carrier fish would not be identified through visual inspection.
- Reproductive material infected or contaminated with typical *A. salmonicida* is unlikely to be detected during inspection because there would be no clinical signs.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of typical *A. salmonicida* was estimated to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

### 7.3.2 Exposure assessment

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

- Typical *A. salmonicida* can be transmitted horizontally via water, contaminated equipment and direct contact between fish.
- Any viable typical *A. salmonicida* which enter the environment would be capable of persisting as free-living bacteria for 2–63 days ((McCarthy 1977) cited in (Rose 1990))(Allen-Austin, Austin & Colwell 1984; Morgan, Cranwell & Pickup 1991)((Hiney 1994) cited in (Agriculture 2009)).
- Typical *A. salmonicida* would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- Species susceptible to typical *A. salmonicida* infection are present in Australia including salmonids, *Anguilla* species and *P. fluviatilis*.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are susceptible to typical *A. salmonicida*.
- Sturgeon is typically cultured in water between 15–20°C (Castellano et al. 2017; Mohler 2003), which is within the water temperature range that correlates with clinical signs of furunculosis (15–20°C) (Lillehaug, Lunestad & Grave 2003; Sako & Hara 1981).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as, typical *A. salmonicida*-infected sturgeon would be exposed to viable typical *A. salmonicida*.
- Introduction into the wild may occur by direct release of imported live sturgeon or its associated wastes from the aquaculture facility into natural waters. This would be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for fish escapes or waste management that would exclude typical *A. salmonicida* from discharges.
- Wild susceptible species may be less abundant than susceptible species in aquaculture facilities. Despite this, wild susceptible species would be expected to be exposed to typical *A. salmonicida* released into natural waters due to its moderate host range in Australian waters.

## Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to typical *A. salmonicida* in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

The partial likelihood of exposure to each exposure group for typical *A. salmonicida* in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**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 typical *A. salmonicida* in **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

The partial annual likelihood of entry and exposure of each exposure group to typical *A. salmonicida* in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very 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 typical *A. salmonicida* were that:

- Typical *A. salmonicida* can be transmitted horizontally via water, contaminated equipment and direct contact between fish. Transmission from broodstock to progeny may also occur.
- Typical *A. salmonicida* can remain infectious in the environment for an extended time, particularly in low-temperature waters and in sediment.
- It is expected that susceptible species in direct contact with typical *A. salmonicida*-infected fish would receive an infectious dose.
- Fish that survive typical *A. salmonicida* infections can remain carriers and become sources of the bacteria.
- Aquaculture and wild fish species present in Australia are susceptible to typical *A. salmonicida*, including salmonids, *Anguilla* species, *Labridae* species and *P. fluviatilis*. Sea lice, bivalves and marine plankton are also present and may act as vectors for typical *A. salmonicida*.
- Furunculosis can be treated by antibiotics although antibiotic-resistance can occur. Trimethoprim (Dihydrofolate Inhibitor) and Oxytetracycline (Tetracycline) are used in Tasmania's salmonid aquaculture industry (Aquaculture 2021).
- Vaccines to control for typical *A. salmonicida* are available but would need to be approved for use in the Australian sturgeon and salmonid industries (Midtlyng 2014).
- The likelihood of typical *A. salmonicida* establishment, following a given quantity of typical *A. salmonicida* entering the environment of an exposure group, is greatest for farmed susceptible species. This is due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and culture conditions.
- Live sturgeon or sturgeon reproductive material could be moved to other aquaculture facilities in Australia. Species polycultured with typical *A. salmonicida*-infected sturgeon or in the same water, could also be moved to other facilities. It is expected that typical *A. salmonicida* would establish in these facilities if present in the animals or reproductive material being translocated.
- Each state and territory have translocation protocols for aquaculture animals, which typically include consideration of typical *A. salmonicida*.
- Spread of typical *A. salmonicida* from farmed to wild susceptible species may occur through the movement of infected polyculture species (e.g. rainbow trout, brown trout and Chinook salmon)



into natural waters to replenish depleted populations (VFA 2022; Water 2023). Typical *A. salmonicida* could be effectively transferred this way because fish may not show clinical signs before relocation.

- If typical *A. salmonicida* were to establish on a farm it could spread to neighbouring farms and wild populations through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of typical *A. salmonicida* be suspected and response measures initiated immediately. However, typical *A. salmonicida* is effectively transmitted through water and can persist in the environment as a free-living bacterium, and farms which share a common water source, such as salmon sea farms, or equipment with an infected population may be exposed to typical *A. salmonicida*.
- The likelihood of typical *A. salmonicida* spread from farms to wild populations or neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however typical *A. salmonicida* could spread this way. Typical *A. salmonicida* could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.
- If one or more index cases of typical *A. salmonicida* 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 typical *A. salmonicida* can survive in the environment as a free-living bacterium, it could persist until susceptible hosts were to encounter it. Further, clinically affected wild animals may be eaten by susceptible animals contributing to transmission.
- The likelihood of typical *A. salmonicida* in a wild population spreading to its natural geographic limits is less than for other hazards with wide host ranges, for example, frog virus 3, but would be more likely than for those hazards which cannot survive outside of a host for long periods. The ability of fish to be subclinically infected with typical *A. salmonicida* and to remain carriers after surviving an infection also aids its spread.
- If typical *A. salmonicida* were to establish in the wild, especially in waters around aquaculture facilities, it may easily spread to farms through water intake due to typical *A. salmonicida* being able to survive as a free-living bacterium and being transmissible through water. In the absence of effective biosecurity measures, wild infected fish or vectors may be transferred into the farms through the inlet water channels.
- If typical *A. salmonicida* were to establish in salmonid aquaculture facilities in Australia then the summer water temperatures in both freshwater and saltwater where salmonids are farmed would favour the spread of typical *A. salmonicida*.

## Conclusion

Based on these considerations and using the descriptors in Table 4, the partial likelihood of establishment and spread of typical *A. salmonicida* in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**Moderate.**
- Wild susceptible species—**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* were that:

#### *Direct effects*

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

- Furunculosis is primarily a disease of farmed salmonids with mortality up to 100% in both natural infections and challenge trials beginning 2–3 days after clinical signs appear. Production and productivity losses due to typical *A. salmonicida* would be significant for the Australian salmonid industry with aquaculture production valued at approximately AU\$1 billion in 2020–21 (Tuynman & Dylewski 2022).
- Morbidity and mortality has also been associated with infection in non-salmonid farmed species such as sturgeon. Production losses in a sturgeon aquaculture industry would also be significant considering sturgeon can take >3 years to reach sexual maturity.
- Typical *A. salmonicida* may impact wild fisheries in Australia. There are reports of typical *A. salmonicida* in wild fish and associated mortalities although no reports of declines in catch rates.
- Based on the impacts of typical *A. salmonicida* infection in salmonid and other fish farming industries in Europe, 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*

- Typical *A. salmonicida* has a moderate host range, and there are reports of serious effects of typical *A. salmonicida* infection in wild fish populations overseas. Although wild fish can also be subclinically infected or carriers of typical *A. salmonicida* rather than clinically affected.
- 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 WOAHA but is included on Australia's National list of reportable diseases of aquatic animals (AHC 2020). State and territory governments would be expected to report on the presence of typical *A. salmonicida*.
- If typical *A. salmonicida* was confirmed at a farm, then an attempt at eradication may be undertaken, which is the preferred response strategy (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 typical *A. salmonicida* was confirmed in the environment, eradication would be near impossible as the agent is able to persist in water and sediments.

- If a movement restricted area were put in place for an outbreak of furunculosis, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- 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 seafood suppliers, commercial wild catch fisheries and bait fisheries due to the moderate host range of typical *A. salmonicida*.
- 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.
- 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*

- Furunculosis is not a WOA-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to typical *A. salmonicida* to avoid the possible introduction of typical *A. salmonicida*. There may also be import requirements or loss of markets for fish products that have been treated with antibiotics or vaccines to prevent typical *A. salmonicida*.
- If typical *A. salmonicida* were to become established, Australia could use zoning to maintain or gain access to international markets for live susceptible species, including sturgeon and non-viable finfish products.
- 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 susceptible 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 typical *A. salmonicida*.

- The impacts of typical *A. salmonicida* 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 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 9 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 9 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
	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 and territory	Minor	D
	Economic (international trade effects)	District or region	Minor	C
	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)	District or region	Minor	C

### Conclusion

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

### 7.3.5 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:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Moderate**.

### 7.3.6 Determination of the partial annual risk

The partial annual risk of typical *A. salmonicida* entry, establishment and spread from **imported live sturgeon** 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Very low**.

The partial annual risk of typical *A. salmonicida* entry, establishment and spread from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Very 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 typical *A. salmonicida* was found to be:

- Imported live sturgeon—**Moderate**.
- Imported sturgeon reproductive material—**Moderate**.

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

## 7.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for typical *A. salmonicida* in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 7.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 10 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of typical *A. salmonicida* but which on their own do not achieve Australia's ALOP for typical *A. salmonicida* in imported live sturgeon or their reproductive material.

**Table 10 Biosecurity measures that on their own do not achieve Australia's ALOP for typical *A. salmonicida***

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Sourcing from disease-free stocks	<b>Yes:</b> Determination of typical <i>A. salmonicida</i> freedom would be to a standard consistent with that recommended for World Organisation for Animal Health (WOAH) listed diseases, or equivalent.
2	Post-arrival quarantine	<b>Yes:</b> The stress of transport can induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
3	Post-arrival batch testing	<b>Yes:</b> Bacterial culture in combination with biochemical testing (Austin & Austin 2012; Cipriano & Bullock 2001) and real-time PCR testing (Bartkova et al. 2017; Byers et al. 2002; Byers, Gudkovs & Crane 2002; Keeling et al. 2013) can detect typical <i>A. salmonicida</i> . Under this scenario, testing is conducted under departmental control and oversight.

#### **7.4.2 Biosecurity measures that in combination achieve Australia's ALOP**

A combination of biosecurity measures 1, 2 and 3 when applied to **imported live sturgeon** would reduce the likelihood of entry of typical *A. salmonicida* from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1, 2 and 3 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of typical *A. salmonicida* from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

## 8 *Argulus alosae*, *Argulus coregoni*, *Argulus flavescens*, *Argulus foliaceus* and *Argulus stizostethii*

### 8.1 Background

*Argulus alosae*, *Argulus coregoni*, *Argulus flavescens*, *Argulus foliaceus* and *Argulus stizostethii* (commonly referred to as fish lice) are aetiological agents of argulosis (Steckler & Yanong 2012). *Argulus* species are in the family *Argulidae* and are widespread crustacean ectoparasites of freshwater and marine fish (Walker et al. 2011). *A. alosae*, *A. coregoni*, *A. flavescens*, *A. foliaceus* and *A. stizostethii* have been detected in Asia, Europe and North America (Neethling & Avenant-Oldewage 2016).

Infection with *Argulus* species is not listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (OIE 2020) and is not on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). Australia has a long history of passive surveillance and a strong system in place to detect incursions. Some species of *Argulus* are known to occur in Australia, including *Argulus japonicus* reported from *Carassius auratus* (goldfish), *Argulus macropterus* from a *Mugil* sp. (mullet), *Argulus australiensis* and *Argulus diversicolor* from *Acanthopagrus* sp. (bream) and *Argulus* sp. A from the introduced *Oreochromis mossambicus* (Mozambique tilapia) (Byrnes 1985; Heegaard 1962; Webb 2008). However, *A. alosae*, *A. coregoni*, *A. flavescens*, *A. foliaceus* and *A. stizostethii* are considered exotic to Australia.

### 8.2 Technical information

#### 8.2.1 Agent properties

*Argulus* species are obligate ectoparasites that have a direct life cycle using fish as hosts (Lester & Roubal 1995). The speed of the life cycle depends upon the species and temperature but is on average 30–60 days with peak abundance typically in summer and autumn (Noga 2000). All life stages of both sexes are parasitic (Steckler & Yanong 2012). Adult *A. coregoni* can reach 12 mm in length whereas *A. foliaceus* adults are smaller at 6–8 mm (Gurney 1948). *A. alosae* adults are 6–12 mm, *A. stizostethii* adults reach 8.5–12 mm and *A. flavescens* adults are the smallest at 4–6 mm (Kabata 1988). Adult male and female *Argulus* mate on or off the host and females then lay several hundred eggs on vegetation and various inert objects in the environment (Taylor, Sommerville & Wootten 2005). *Argulus* can generate eggs multiple times within a year such as *A. foliaceus*, which can have up to 3 generations of egg hatching per year and *A. coregoni* with 2 generations (Taylor, Sommerville & Wootten 2005).

Eggs usually hatch within 10–50 days with the hatching period temperature dependent (Paperna 1991). For example, at 23°C *A. foliaceus* eggs can take 17 days to hatch, whereas at 9–13.9°C they may require a number of months to hatch (Mikheev et al. 2001; Steckler & Yanong 2012). Eggs laid in autumn can survive over winter under certain environmental conditions and hatch in spring (Bower-Shore 1940; Shimura 1983). It was also demonstrated that eggs of *A. coregoni* are able to remain viable under low temperature conditions for up to 2 years (Mikheev et al. 2001). Hatched larvae

must typically find a host within 2–3 days or they will die (Roberts 2001). Once attached to the fish host, juveniles undergo a series of molts (e.g. 11 molts or 12 stages in *A. foliaceus*) until they reach sexual maturity, roughly 30–40 days after hatching (Steckler & Yanong 2012).

*Argulus* attach to the body of the fish host by means of suckers and hooks and feed on host tissue as well as being obligate blood feeders (Walker et al. 2011). They survive on fish hosts for several months. For example, *A. foliaceus* grew on *Cyprinus carpio* (carp) and *Gasterosteus* species (stickleback) for over 6 months (Bower-Shore 1940). *A. foliaceus* have also been found to remain on dead fish hosts for up to 8 days (Bower-Shore 1940). *Argulus* do not remain in contact with the same host. They may become dislodged by the fish or detach themselves for the purpose of mate location, egg deposition or to locate a more preferable host (Mikheev, Pasternak & Valtonen 2007; Walker et al. 2011). *Argulus* are strong swimmers and can survive for several days independent of a fish host (Noga 2000). The off-host survival time varies depending on the life cycle stage and water temperature. For example, larval *A. foliaceus* survived off-host for up to 5 days at 15°C, adults up to 14 days at 9°C and juveniles up to 7 days at 9°C and 15°C (Walker et al. 2011).

*Argulus* juveniles and adults are sensitive to chemical treatments including emamectin benzoate, liflubenzuron, lufenuron, potassium permanganate and trichlorfon (Hakalahti-Sirén, Mikheev & Valtonen 2008; Noga 2000; Steckler & Yanong 2012). Egg hatching can be suppressed by drying for a minimum of 24 hours and with formalin treatment (120 mL/L) (Hakalahti-Sirén, Mikheev & Valtonen 2008).

## 8.2.2 Epidemiology

### Host range

Species which are reported to be susceptible to infestation (N= natural exposure; E= experimental exposure) with *A. alosae*, *A. coregoni*, *A. flavescens*, *A. foliaceus* and *A. stizostethii* include but are not limited to:

- *Abramis brama*<sup>N</sup> (bream) – *A. foliaceus* (Gurney 1948)
- *Acipenser brevirostrum*<sup>N</sup> (shortnose sturgeon) – *A. alosae* (Appy & Dadswell 1978)
- *Acipenser fulvescens*<sup>N</sup> (lake sturgeon) – *A. stizostethii* (Cressey 1978)
- *Acipenser oxyrinchus*<sup>N</sup> (Atlantic sturgeon) – *A. coregoni*, *A. stizostethii* (Munroe et al. 2011; Popielarczyk & Kolman 2013)
- *Acipenser oxyrinchus desotoi*<sup>N</sup> (Gulf sturgeon) – *A. flavescens* (Andres et al. 2019)
- *Acipenser* sp.<sup>N</sup> (species not specified) – *A. foliaceus* ((Ivanova, Golovina & Golovin 1993) cited in (Bauer, Pugachev & Voronin 2002))
- *Alburnus alburnus*<sup>N</sup> (bleak) – *A. foliaceus* (Dzika, Kuszczala & Kozłowski 2008)
- *Alosa pseudoharengus*<sup>N</sup> (alewife) – *A. alosae* (Cressey 1978)
- *Ameiurus natalis*<sup>N</sup> (yellow bullhead) – *A. flavescens* ((Wilson 1916) cited in (Neethling & Avenant-Oldewage 2016))
- *Amia calva*<sup>N</sup> (bowfin) – *A. flavescens* ((Bangham 1940) cited in (Neethling & Avenant-Oldewage 2016))



- *Astronotus ocellatus*<sup>N</sup> (tiger oscar) – *A. foliaceus* (Toksen 2006)
- *Carassius auratus*<sup>N</sup> (goldfish) – *A. foliaceus* (Noaman, Chelongar & Shahmoradi 2010)
- *Carassius carassius*<sup>N</sup> (crucian carp) – *A. foliaceus* (Paperna 1991)
- *Clupea harengus*<sup>N</sup> (Atlantic herring) – *A. alosae* (Cressey 1978)
- *Coregonus artedii*<sup>N</sup> (cisco) – *A. stizostethii* (Kabata 1988)
- *Coregonus lavaretus*<sup>N</sup> (European whitefish) – *A. coregoni* ((Thorell 1864) cited in (Neethling & Avenant-Oldewage 2016))
- *Ctenopharyngodon idella*<sup>N</sup> (grass carp) – *A. foliaceus* (Oktener et al. 2006)
- *Cyprinus carpio*<sup>N</sup> (carp, common carp, koi carp) – *A. flavescens*, *A. foliaceus* (Gurney 1948; Oktener et al. 2006; Roberts 1957)
- *Dorosoma cepedianum*<sup>N</sup> (American gizzard shad) – *A. stizostethii* (Cressey 1978)
- *Esox lucius*<sup>N</sup> (pike) – *A. foliaceus* (Gurney 1948)
- *Esox masquinongy*<sup>N</sup> (Muskellunge) – *A. stizostethii* (Cressey 1978)
- *Huso huso*<sup>N</sup> (beluga) – *A. coregoni*, *A. foliaceus* (Scanzio & Prearo 2012)
- *Gasterosteus aculeatus*<sup>N, E</sup> (three spined stickleback) – *A. foliaceus*, *A. stizostethii* (Bower-Shore 1940; Kabata 1988; Poulin & Fitzgerald 1987)
- *Gobio gobio*<sup>N</sup> (gudgeon) – *A. foliaceus* (Bower-Shore 1940)
- *Ictalurus punctatus*<sup>N</sup> (channel catfish) – *A. flavescens* (Poly 1998)
- *Mastacembelus mastacembelus*<sup>N</sup> (spiny eel) – *A. foliaceus* (Oktener et al. 2006)
- *Microgadus tomcod*<sup>N</sup> (Atlantic tomcod) – *A. alosae* (Cressey 1978)
- *Micropterus salmoides*<sup>N</sup> (largemouth black bass) – *A. flavescens* (Poly 1998)
- *Oncorhynchus masou*<sup>N</sup> (Masou salmon) – *A. coregoni* (Shimura et al. 1983)
- *Oncorhynchus masou ishikawae*<sup>N</sup> (amago salmon) – *A. coregoni* (Nagasawa & Yuasa 2020)
- *Oncorhynchus mykiss*<sup>N</sup> (rainbow trout) – *A. coregoni*, *A. foliaceus* (Buchmann & Bresciani 1997; Mikheev, Pasternak & Valtonen 2007; Shimura 1983)
- *Oreochromis mossambicus* × *Oreochromis niloticus*<sup>N</sup> (red tilapia) – *A. coregoni* (Everts & Avenant-Oldewage 2009)
- *Perca fluviatilis*<sup>N</sup> (European perch, redfin) – *A. foliaceus*, *A. stizostethii* (Bower-Shore 1940; Gurney 1948; Kabata 1988)
- *Planiliza abu*<sup>N</sup> (Abu mullet) – *A. foliaceus* (Oktener et al. 2006)
- *Pylodictis olivaris*<sup>N</sup> (flathead catfish) – *A. flavescens* (Andres et al. 2019)
- *Pungitius pungitius*<sup>N, E</sup> (ninespine stickleback) – *A. stizostethii* (Poulin & Fitzgerald 1987)
- *Rutilus rutilus*<sup>N</sup> (roach) – *A. coregoni*, *A. foliaceus* (Bower-Shore 1940; Northcott, Lyndon & Campbell 1997)

- *Salmo salar*<sup>N</sup> (Atlantic salmon) – *A. foliaceus*, *A. stizostethii* (Bower-Shore 1940; Kabata 1988)
- *Salmo trutta*<sup>N</sup> (brown trout) – *A. coregoni*, *A. foliaceus* (Bower-Shore 1940; Gurney 1948)
- *Salvelinus fontinalis*<sup>N</sup> (brook trout) – *A. coregoni*, *A. stizostethii* ((Hoshina 1950) cited in (Shimura 1983))(Cressey 1978)
- *Sander vitreus*<sup>N</sup> (walleye) – *A. stizostethii* (Cressey 1978)
- *Scardinius erythrophthalmus*<sup>N</sup> (rudd) – *A. foliaceus* (Oktener et al. 2006)
- *Silurus triostegus*<sup>N</sup> (tigris catfish) – *A. foliaceus* (Oktener et al. 2006)
- *Strongylura marina*<sup>N</sup> (Atlantic needlefish) – *A. alosae* (Cressey 1978)
- *Thymallus thymallus*<sup>N</sup> (Grayling) – *A. coregoni*, *A. foliaceus* (Bower-Shore 1940; Neethling & Avenant-Oldewage 2016)
- *Tinca tinca*<sup>N</sup> (tench) – *A. foliaceus* (Bower-Shore 1940; Oktener et al. 2006)
- *Triturus vulgaris vulgaris*<sup>N</sup> (smooth newt) – *A. foliaceus* (Bower-Shore 1940).

*Argulus* infests all life stages of fish, particularly juvenile fish, except for eggs (Hogans 1994).

*A. coregoni* infested *A. oxyrinchus oxyrinchus* with weight 11–74 kg (Popielarczyk & Kolman 2013) and *A. flavescens* infested juvenile and adult *A. oxyrinchus desotoi* (Andres et al. 2019).

### Geographical distribution

*A. alosae*, *A. coregoni*, *A. flavescens*, *A. foliaceus* and *A. stizostethii* have been reported in Canada (Popielarczyk & Kolman 2013), China ((Wang 1958) cited in (Shimura 1983)), Denmark (Buchmann, Uldal & Lyholt 1995), Finland (Pasternak, Mikheev & Valtonen 2004), France ((Laurent 1975) cited in (Neethling & Avenant-Oldewage 2016)), Iran (Noaman, Chelongar & Shahmoradi 2010), Japan (Shimura 1983), Malaysia (Everts & Avenant-Oldewage 2009), Poland (Popielarczyk & Kolman 2013), Portugal (Menezes et al. 1990), Republic of Macedonia (Blazhekovikj-Dimovska, Doligalska & Nowakowska 2017), Republic of Türkiye (Cengizler et al. 2001), Slovakia (Aalberg et al. 2016), Sweden (Neethling & Avenant-Oldewage 2016), United Kingdom (UK) (Gurney 1948; Taylor, Sommerville & Wootten 2005) and United States of America (USA) (Andres et al. 2019; Hogans 1994).

### Prevalence

#### *Sturgeon*

In 2002, *A. fulvescens* (lake sturgeon) were captured in St Mary River, USA and 22% (n=45) were infested with *A. stizostethii* or *A. coregoni* (CGLBLSG 2004). *A. stizostethii* was present at a prevalence of 8% on *A. oxyrinchus* collected from commercial fish weirs in the Bay of Fundy, Canada in 2009 (n=26) (Munroe et al. 2011). During 2016–2019, *A. oxyrinchus desotoi* captured in the Pascagoula River, Missouri, USA were positive for *A. flavescens* at a prevalence of 70.8% (n=209) (Andres et al. 2019).

#### *Salmonids*

In the UK, 29% of the trout fishing sites tested in 2000 (n=70) were positive for *A. foliaceus* (Taylor, Sommerville & Wootten 2005). *A. coregoni* prevalence in a *O. mykiss* farm in Finland in 2002 was 100% (n=75) (Bandilla et al. 2005). *A. coregoni* prevalence of 35% (n=20) was detected on *O. masou ishikawae* from a farm in Shikoku, Japan in 2019 (Nagasawa & Yuasa 2020).

### Other fish

A survey of fish collected from Lake Kortowskie, Poland in 2001–2004 reported an *A. foliaceus* prevalence of 3.57% on *P. fluviatilis* (n=56), 2.38% on *A. alburnus* (n=46) and 16.66% on *E. lucius* (n=6) (Dzika, Kuszala & Kozłowski 2008). Sampling of fish in a commercial fishery in England in 2002 detected *A. foliaceus* on 8.5% of *S. erythrophthalmus* (n=153), 40.5% *A. brama* (n=42), 41.7% *T. tinca* (n=24), 23.5% *C. carassius* (n=34) and 65.7% *C. carpio carpio* (n=140) (Walker et al. 2008). The prevalence of *A. foliaceus* was 1.3% on *L. abu* (n=155), 14.3% on *M. mastacembelus* (n=14) and 100% on *S. triostegus* (n=1) caught from Atatürk Dam Lake, Republic of Türkiye in 2006 (Oktener et al. 2006). In 2008, *A. foliaceus* was detected at a prevalence of 75% (n=80) on a *C. auratus* farm in Iran (Noaman, Chelongar & Shahmoradi 2010). A survey carried out on *C. carpio* aquaculture facilities in the Republic of Macedonia during 2009–2013 reported *A. foliaceus* at a prevalence of 6% (n=958) (Blazhekovikj-Dimovska, Doligalska & Nowakowska 2017). *S. fontinalis* and *S. lucioperca* were sampled from culture ponds in Slovakia during 2013–2015 and *A. foliaceus* was detected at 100% (n=2) and 80–100% (n=40), respectively (Aalberg et al. 2016). In district D.I. Khan Khyber Pakhtunkhwa, Pakistan, 10% (n=150) of *C. carpio* were infested with *A. foliaceus* and 6.66% (n=150) with *A. coregoni* in 2014 (Khan et al. 2017).

### Mortalities

Heavy infestations of *Argulus* can cause mass mortalities, especially in juvenile fish (Hogans 1994; Noaman, Chelongar & Shahmoradi 2010; Taylor, Sommerville & Wootten 2005). There are limited publications about mortalities in wild or farmed populations due to *Argulus*.

### Sturgeon

*A. foliaceus* reportedly caused mortality of sturgeon in farms of the Azov River basin (no numbers given) ((Ivanova, Golovina & Golovin 1993) cited in (Bauer, Pugachev & Voronin 2002)).

### Salmonids

Massive mortalities (no numbers given) were observed in *A. foliaceus*-infested *O. mykiss* cultured in floating cages in the Azores in 1988 when the fish were aged 1 year (weight 300 g) (Menezes et al. 1990). A *O. mykiss* fishery located near Bangor, Northern Ireland, is reported to have had *A. foliaceus* outbreaks resulting in massive fish kills in 1995, 1999 and 2002 (no numbers given) (Harrison, Gault & Dick 2006).

### Other fish

*C. carpio* infested with *A. foliaceus* on a farm in the Republic of Türkiye suffered mass mortalities (no numbers given) (Pekmezci et al. 2011). Rahman (1996) found over 80% of *C. carpio* (1.5–2 cm) were killed within 24 hours when experimentally infested with 20–30 *A. foliaceus* ((Rahman 1996) cited in (Taylor, Sommerville & Wootten 2005)).

### Transmission

*Argulus* can be transmitted via contact between fish or water (Steckler & Yanong 2012). Initial infestation of cultured fish is typically caused by introduction of parasitised hosts (Hogans 1994). Water transmission is dependent upon the off-host survival time of the parasites and environmental conditions (temperature) (Paperna 1991; Walker et al. 2011).

Contaminated sediment and fomites may contribute to transmission. For example, marine sand containing live *A. foliaceus* eggs is suspected as the source of an outbreak on a *C. auratus* farm in Iran (Noaman, Chelongar & Shahmoradi 2010). Stressors such as high host density, low dissolved oxygen

levels and slow current conditions enhance the spread of the disease (Hogans 1994; Poulin & Fitzgerald 1987). The wide geographic distribution and host range of *Argulus* indicates the ability for these parasites to rapidly spread (Walker et al. 2011).

*Argulus* are known to act as vectors for other disease agents that can cause secondary infections in the fish (Bower-Shore 1940; Shimura 1983). For example, Pfeil-Putzien & Baath (1977) isolated spring viraemia of carp virus (SVCV) from *A. foliaceus* and showed that it could transfer the virus to carp (Pfeil-Putzien 1977). Typical *Aeromonas salmonicida*, the aetiological agent of furunculosis, has been isolated from *A. coregoni* (Shimura 1983).

### 8.2.3 Pathogenesis

#### Tissue tropism

*Argulus* are found predominantly on the skin, fins, gills or mouth of fish (Andres et al. 2019; Hogans 1994; Steckler & Yanong 2012).

#### Tissue titre

*A. coregoni* was observed on the skin of *A. oxyrinchus* captured from St John River in Canada at 1–2 parasites per fish (Popielarczyk & Kolman 2013). *A. foliaceus* reportedly caused mortality of sturgeon in farms of the Azov River basin with up to 15 parasites per fish (age one-summer-old) (Ivanova, Golovina & Golovin 1993) cited in (Bauer, Pugachev & Voronin 2002)). *A. oxyrinchus desotoi* captured in the Pascagoula River, USA were infested with 1–64 individual *A. flavescens* (Andres et al. 2019).

Diseased *O. mykiss* from an aquaculture lake system that suffered mass mortalities reportedly carried several tens of *A. foliaceus* per fish on gills, skin and mouth cavity (Menezes et al. 1990). Attached *A. coregoni* on *O. mykiss* in an infected farm in Finland ranged from 4–1390 individuals per fish (Bandilla et al. 2005). Up to 1500 *A. foliaceus*, mostly juvenile stages, were reported on a single *O. mykiss* during an outbreak in a fishery in Scotland (Northcott, Lyndon & Campbell 1997). The number of *A. foliaceus* noted per fish at the peak of infestation of trout (species not specified) in UK fisheries ranged from <10 to >100 (Taylor, Sommerville & Wootton 2006). A survey to determine the population of *A. coregoni* on farmed *O. mykiss* over time reported that the number of parasites per fish ranged from approximately 30 in June–July (summer) to 1.8 in August to 0.05 in September–October (Hakalahti & Valtonen 2003).

In the same lake, the maximum number of parasites on other fish sampled were 23 on *C. carpio*, 9 on *E. lucius* and 6 on *P. fluviatilis* (Menezes et al. 1990). Pekmezci et al. (2011) reported a maximum of 800–1000 *A. foliaceus* per individual *C. carpio* causing mass mortality in a fish farm in the Republic of Türkiye (Pekmezci et al. 2011). The mean number of *A. foliaceus* on infested farmed *C. auratus* in Iran was 2–3 per fish (Noaman, Chelongar & Shahmoradi 2010).

### 8.2.4 Diagnosis

#### Clinical signs

One or two parasites usually cause no clinical signs in large fish, but *Argulus* have a high reproductive rate, often resulting in rapid increase in infestations (Noga 2000; Steckler & Yanong 2012). *Argulus* feed by inserting a pre-oral sting (stylet) into the fish host and sucking body fluids with the proboscis-like mouth (Noga 2000). Fish are damaged by the repeated piercing of the skin by the stylet, which injects toxic enzymes, causing irritation (Noga 2000). Hooks and spines on the parasite's appendages also cause mechanical damage (Kabata 1988). Other clinical signs include reduced

feeding, lethargy, poor body condition, pale gills and a loss of equilibrium (Menezes et al. 1990; Taylor, Sommerville & Wootten 2005). Fish are reported to jump, flash and swim erratically, possibly in an attempt to rid themselves of the parasites, and to swim in tight shoals (Menezes et al. 1990; Taylor, Sommerville & Wootten 2005). Mortalities are usually associated with severe infestations, for example when there are hundreds of *Argulus* per fish (Shimura 1983).

### **Pathology**

Focal necrosis and haemorrhage may develop at the point of stylet entry (Hogans 1994; Menezes et al. 1990). Secondary fungal and bacterial infections can then develop in the lesions (Bower-Shore 1940; Kumar et al. 2017; Northcott, Lyndon & Campbell 1997). Severe infestations may lead to excessive mucus production, anaemia and loss of ionic and osmotic homeostasis (Kumar et al. 2017; Steckler & Yanong 2012).

### **Testing/detection**

Adult *Argulus* are visible to the naked eye but their colour and patterning may hinder visual detection. Diagnosis is made by morphological identification of the parasites (Kabata 1988). The parasite frequently moves on a host and may be seen swimming in the water when they are in a farm (Noga 2000).

## **8.2.5 Treatment**

Individual parasites can be removed from fish with forceps, but this can be time consuming, smaller individuals might be missed and does not eliminate parasites in the environment (Noaman, Chelongar & Shahmoradi 2010). Mechanical treatments to remove *Argulus* from fish such as shaking in a net, hydrolicers and thermolicers can also be used (Fletcher 2019).

Chemicals treatments have been used for argulosis but chemical resistance, availability, legality of use on food fish species, fish species sensitivity, impact on the environment, dosage rates and costs must be taken into account (Hakalahti-Sirén, Mikheev & Valtonen 2008; Kumar et al. 2017; Steckler & Yanong 2012). Repeated treatments may be required if the chemical is only effective against one life cycle stage to ensure all parasites are eradicated (Paperna 1991). Trichlorfon and emamectin benzoate have been shown to be effective at treating *Argulus* infestations (Hakalahti, Pasternak & Valtonen 2004; Hanson et al. 2011; Steckler & Yanong 2012). Formaldehyde, potassium permanganate and lufenuron are also treatment options (Hakalahti-Sirén, Mikheev & Valtonen 2008; Mayer et al. 2013; Noaman, Chelongar & Shahmoradi 2010).

## **8.2.6 Control and prevention**

*Argulus* eggs are sensitive to drying and formalin treatments (Hakalahti-Sirén, Mikheev & Valtonen 2008). Therefore, fallowing farm sites between successive stockings, draining and drying egg laying sites and using disinfectant on tanks can remove egg contamination (Hakalahti-Sirén, Mikheev & Valtonen 2008; Hakalahti, Pasternak & Valtonen 2004; Noga 2000). Installing artificial substrates in ponds on which the *Argulus* can lay its eggs that can be removed and cleaned during spawning is another control measure used (Gault, Kilpatrick & Stewart 2002; Mikheev, Pasternak & Valtonen 2015). Other management practices to control infestation of ponds include removing suitable egg-laying substrata, using filtered water or water obtained from a *Argulus*-free source, stocking fish at low densities, timing of seasonal activities and introducing fish that feed on adult parasites (Kumar et al. 2017; Lester & Roubal 1995). It is also recommended that incoming fish should be quarantined,

observed, and inspected in order to minimise the risk of introduction of *Argulus* into the aquaculture facility (Steckler & Yanong 2012).

### 8.2.7 Impact of the disease

Fish lice parasitism is considered one of the more serious threats to farmed fish in warm water systems (Paperna 1991). Losses due to *Argulus* infestations result from morbidity and mortality of affected fish due to the parasites and the associated secondary infections (Noaman, Chelongar & Shahmoradi 2010). In aquaculture facilities, this can lead to unprofitable harvests. For example, the economic cost to carp culture ponds in India due to argulosis, considering mortality occurred, loss in growth and expenditure towards drugs and chemicals was estimated to be US\$615 per hectare per year (Sahoo et al. 2013).

### 8.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon or their reproductive material as import is not permitted.

There are biosecurity measures to manage the risk of *A. coregoni* and *A. foliaceus* on imported ornamental fish for display purposes (see [Appendix D](#)).

## 8.3 Risk assessment

Based on [chapter 4](#) and the technical information about *Argulus* presented in this chapter, a risk assessment was completed. For simplicity, the *Argulus* species retained as hazards (*A. alosae*, *A. coregoni*, *A. flavescens*, *A. foliaceus* and *A. stizostethii*) will be collectively referred to as *Argulus*.

A summary of the risk assessment values for determining if the overall annual risk of *Argulus* species meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#).

### 8.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that *Argulus* are present in all source countries.
- *Argulus* infest live sturgeon that would be of a life stage exported to Australia.
- Prevalence of *A. coregoni*, *A. flavescens* and *A. stizostethii* up to 70% have been reported in wild sturgeon (Andres et al. 2019; CGLBSG 2004; Munroe et al. 2011). There is one report of *A. stizostethii* in farmed sturgeon at a prevalence of 8% (Munroe et al. 2011).
- Prevalence of *Argulus* in other farmed fish can reach 100% and in wild populations can be up to 30%.
- Sturgeon reproductive material is not expected to be infested with the ectoparasites as *Argulus* attach and feed on fish after they hatch.
- All stages of the *Argulus* life cycle can survive independently of the fish host.
- Adult *Argulus* are likely to be detected on live sturgeon during inspection. However, low-level infestations, infestations of young parasites and fish showing mild or no clinical signs would be unlikely to be detected.

- *Argulus* have a high reproductive rate that can result in rapid escalation of infestations.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of *Argulus* was estimated to be:

- Imported live sturgeon—**Moderate**.
- Imported sturgeon reproductive material—**Negligible**.

Because the likelihood entry for *Argulus* on sturgeon reproductive material was estimated to be negligible, the risk assessment was stopped, and only live sturgeon were considered further.

### 8.3.2 Exposure assessment

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

- *Argulus* can be transmitted via contact between fish and through water.
- *Argulus* are capable of surviving for a short period as free-living parasites.
- *Argulus* have a high reproductive rate that can result in rapid escalation of infestations.
- Species susceptible to *Argulus* infestation are present in Australia including *A. ocellatus*, *C. auratus*, *C. carpio*, *O. mossambicus*, *P. fluviatilis*, *R. rutilus*, *Strongylura* species, *T. tinca* and salmonids.
- Aquaculture species most likely to be polycultured with imported sturgeon such as trout are susceptible to *Argulus*.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the temperature range for *Argulus* reproduction (Mikheev et al. 2001).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infested sturgeon will be certain to be exposed to viable *Argulus*.
- Introduction into the wild may occur by direct release of imported live sturgeon or its associated wastes from the aquaculture facility into natural waters. This would be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for fish escapes and waste management that would exclude *Argulus* from discharges.
- Wild susceptible species may be less abundant than susceptible species in aquaculture facilities. Despite this, wild susceptible species would be expected to be exposed to *Argulus* released into natural waters due to their wide host range in Australian waters.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to *Argulus* in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Moderate**.



### 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 *Argulus* in **imported live sturgeon** 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**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 *Argulus* were that:

- *Argulus* can be transmitted via contact between fish and through water. They can survive as a free-living parasite in the aquatic environment.
- *Argulus* can survive on fish for several months and spawn several times during this period.
- Aquaculture and wild fish species present in Australia are susceptible to *Argulus* including *A. ocellatus*, *C. auratus*, *C. carpio*, *O. mossambicus*, *P. fluviatilis*, *R. rutilus*, *Strongylura* species, *T. tinca* and salmonids.
- The likelihood of *Argulus* establishment is high for farmed susceptible species. This is due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- If infested live sturgeon or polycultured species in an aquaculture facility were moved to another aquaculture facility in Australia it is likely that *Argulus* would establish in these facilities.
- Each state and territory have translocation protocols for aquaculture animals but some disease agents may not be covered, including *Argulus*.
- There are treatment options for *Argulus* infestations but repeat treatments and careful chemical selection, if available, is required.
- Spread of *Argulus* from farmed to wild susceptible species may occur through the movement of infested polyculture species (e.g. rainbow trout, brown trout and Chinook salmon) into natural waters to replenish depleted populations. *Argulus* could be effectively transferred this way because fish may not show obvious infestation or clinical signs before transfer.
- If *Argulus* were to establish on a farm it could spread to neighbouring farms and wild populations through wastewater. This spread would be moderated by implementation of biosecurity measures should an infestation of *Argulus* be suspected and response measures initiated immediately. However, *Argulus* is effectively transmitted through water and can persist in the environment as a free-living parasite, and farms which share a common water source or equipment with an infested population may be exposed to *Argulus*.
- The likelihood of *Argulus* spread from farms to wild populations or neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however the parasites could spread this way. *Argulus* could also be spread from farms



to wild populations via birds scavenging infested moribund fish and dropping them into unaffected waters.

- If *Argulus* were to establish in salmonid aquaculture facilities then the summer water temperatures where salmonids are farmed would favour the spread of the ectoparasites.
- If one or more index cases of *Argulus* were to occur in the wild, establishment and spread would be similar to on a farm because the wide host range of susceptible animals increases opportunities for transmission. Further, because *Argulus* can survive in the environment as a free-living parasite for a period, it could persist until susceptible hosts were to encounter it.
- The likelihood of *Argulus* in a wild population spreading to its natural geographic limits is greater than for other hazards with moderate host ranges, such as typical *Aeromonas salmonicida*, and would be more likely than for those hazards which cannot survive outside of a host for long periods. The ability of *Argulus* to survive on fish for several months also aids its spread.
- If *Argulus* were to establish in the wild, especially in waters around aquaculture facilities, it may easily spread to farms through water intake due to *Argulus* being able to survive as a free-living parasite and being transmissible through water. In the absence of effective biosecurity measures, wild infected fish and vectors may also move into the farms through inlet water channels.

### Conclusion

Based on these considerations and using the descriptors in Table 4, the partial likelihood of establishment and spread of *Argulus* in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**High**.

### Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of *Argulus* were that:

#### *Direct effects*

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

- Primarily a disease of cyprinids and salmonids but other species susceptible to *Argulus* are present in Australia. Heavy infestations may cause high rates of mortality in farmed juvenile fish, including sturgeon. Although, treatment options for *Argulus* infestations are available.
- Production and productivity losses due to *Argulus* would be significant for the Australian salmonid industry with aquaculture production valued at approximately AU\$1 billion in 2020–21 (Tuynman & Dylewski 2022).
- Production losses in a sturgeon aquaculture industry would be considerable considering sturgeon require >3 years to reach sexual maturity.
- The domestic koi carp industry, estimated to conservatively expend \$20–52 million Australia-wide on production costs (DAFF 2022), would be significantly affected by an outbreak.

- *Argulus* may impact wild fisheries in Australia. There are reports of *Argulus* in wild fish although no reports of associated declines in catch rates.
- Based on the impacts of *Argulus* infestations in salmonid and other fish farming industries, 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*

- Susceptible species are distributed in Australian waters and *Argulus* have been detected in wild fish populations elsewhere in the world. However, there have been no reports of mortalities in the wild due to *Argulus*.
- The direct impact of *Argulus* 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*

- Infestation with *Argulus* is not listed as a notifiable disease by WOAHP nor is it included on Australia's National list of reportable diseases of aquatic animals (AHC 2020). Although not listed in Australia, state and territory governments would be expected to report on the presence of *Argulus*.
- If *Argulus* were confirmed at a farm then attempts at eradication would likely be undertaken. Argulosis can be treated with chemicals and controlled by various management practices. However, repeated treatments would be required to remove all the ectoparasites and its free-living stages and the chemicals must be approved for use in Australia and aquaculture.
- 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 an infestation of *Argulus*, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- If eradication was unsuccessful, then on-going control programs may need to be developed to control outbreaks of *Argulus* in aquaculture facilities.
- If *Argulus* was confirmed in the environment, eradication would be near impossible as all parasite stages can survive independently of the fish host and eggs can be laid on vegetation and various inert objects in the environment.
- Eradication and control of *Argulus* 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 areas put in place would have indirect impacts on other industries such as seafood suppliers and ornamental fish facilities due to the wide host range of *Argulus*.
- 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.

- *Argulus* infested fish may show gross signs which may affect their marketability.
- *Argulus* 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 *Argulus* is not a WOA-listed disease. Importing countries may have import requirements or have closed borders to the import of live species susceptible to *Argulus* to avoid the possible introduction of *Argulus*.
- If *Argulus* were to become established, Australia could use zoning to maintain or gain access to international markets for live fish and, if required, non-viable product.
- The impacts of *Argulus* establishment and spread on international trade is expected to be minor at the local level.

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

- *Argulus* have a wide host range but are not considered to cause significant mortality in wild susceptible fish.
- No endangered Australian fish species are currently known to be susceptible to infestation with *Argulus*.
- The impacts of *Argulus* establishment and spread on biodiversity are 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 *Argulus* are recreationally fished in Australia and could be affected by movement restriction areas put in place due to an outbreak of argulosis which may impact on social amenity. This includes impacts on important species for indigenous cultural fishing, such as perch, snapper and bream.
- Fish infested with *Argulus* also deters recreational fishers either because they are unable to catch fish or because the appearance of the fish is unappealing (Taylor, Sommerville & Wootten 2006).
- In local areas where aquaculture or recreational fishing of susceptible species is a major industry, an argulosis outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of *Argulus* establishment and spread are expected to be minor at the district or regional level.

Table 11 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of *Argulus*. 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 11 Overall impact of establishment and spread of *Argulus* 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)	Local	Minor	B
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)	Local	Minor	B
	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)	District or region	Minor	C

**Conclusion**

The overall impact of establishment and spread of *Argulus* was estimated to be **moderate**.

**8.3.5 Determination of likely consequences of the outbreak scenario**

The likely consequences of the outbreak scenario for *Argulus* 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

**8.3.6 Determination of the partial annual risk**

The partial annual risk of *Argulus* entry, establishment and spread 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

**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 risk associated with *Argulus* was found to be:

- Imported live sturgeon—**Moderate**.

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

## 8.4 Biosecurity measures

Details of the risk assessment values for determining whether biosecurity measures manage the biosecurity risk for *Argulus* in imported live sturgeon to a level that achieves Australia's ALOP are shown in [Appendix B](#).

### 8.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 12 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of *Argulus* but which on their own do not achieve Australia's ALOP for *Argulus* in imported live sturgeon.

**Table 12 Biosecurity measures that on their own do not achieve Australia's ALOP for *Argulus* species**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Disease-free stock	<b>Yes:</b> Determination of <i>Argulus</i> freedom would need to be to a standard consistent with that recommended for World Organisation for Animal Health (WOAH) listed diseases, or equivalent.
2	Pre-export parasite treatment	<b>Yes:</b> <i>Argulus</i> are sensitive to parasite treatment. However, treatments can be ineffective at removing all the parasites so reinfestation can occur.
3	Post-arrival quarantine (PAQ)	<b>Yes:</b> Infested sturgeon can be detected during the PAQ period.
4	Post-arrival parasite treatment	<b>Yes:</b> <i>Argulus</i> are sensitive to parasite treatment. Treatments may need to be repeated to be effective.

### 8.4.2 Biosecurity measures that in combination achieve Australia's ALOP

Either biosecurity measure 1 or 2 in combination with 3 and 4 when applied to **imported live sturgeon** would reduce the likelihood of entry of *Argulus* from **moderate** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

## 9 Cyprinid herpesvirus 3 (Koi herpesvirus)

### 9.1 Background

Cyprinid herpesvirus 3 (CyHV-3), also commonly known as Koi herpesvirus (KHV), is a member of the genus *Cyprinivirus* in the family *Alloherpesviridae* and is the aetiological agent of koi herpesvirus disease (KHVD) (Haramoto et al. 2007; Waltzek et al. 2009). CyHV-3 is primarily a disease of *Cyprinus carpio* (carp species) with mass mortality events occurring in both aquaculture and wild populations (OIE 2021c). Following the first reports of KHVD in Israel, Germany and the United Kingdom in the 1990s, it is now known to occur in, or has been recorded in at least 33 different countries (Australian Government & FRDC 2022; OIE 2021c).

Infection with KHV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (OIE 2021c) and is on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). Australia has a long history of passive surveillance and a strong system in place to detect incursions. CyHV-3 is considered exotic to Australia. However, CyHV-3 is currently being considered as a biological control agent for carp in Australia (Australian Government & FRDC 2022).

### 9.2 Technical information

#### 9.2.1 Agent properties

CyHV-3 is an icosahedral, enveloped, double-stranded DNA virus (170–230 nm) (Ilouze et al. 2011; Waltzek et al. 2009) that is formally classified by the International Committee on Taxonomy of Viruses as a member of the genus *Cyprinivirus* in the family *Alloherpesviridae* (ICTV 2020). Waltzek et al. (2005) provided evidence to support the classification of the virus as a herpesvirus and named it cyprinid herpesvirus-3 (CyHV-3), following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, fish papilloma virus) and CyHV-2 (goldfish haematopoietic necrosis virus) (Waltzek et al. 2005). CyHV-3 has also been referred to as carp interstitial nephritis and gill necrosis virus (CNGV) and koi herpes-like virus (Dishon et al. 2005; Waltzek et al. 2009).

Outbreaks of KHVD typically occur between 16–28°C (Haenen et al. 2004; Hedrick et al. 2000). Mortality events due to KHVD have been reported to increase when the temperature reaches 22–26°C (Perelberg et al. 2003). Under experimental conditions, the disease has caused high mortality at 28°C but not at 29°C or at 13°C (Gilad et al. 2004; Ronen et al. 2003). However, viral DNA was detected in fish by PCR at 13°C, indicating infected fish surviving at low temperatures may be reservoirs of the virus (Gilad et al. 2004).

CyHV-3 remains infectious in water for at least 4 hours but not for 21 hours at water temperatures of 23–25°C (Perelberg et al. 2003). In the absence of hosts, free particles of CyHV-3 become quickly inactivated in environmental water, likely by microorganisms (Shimizu et al. 2006). The detection of CyHV-3 DNA in river water samples at temperatures of 9–11°C was reported 4 months before an outbreak of KHVD in the same river (Haramoto et al. 2007). However, persistence of the virus may

have been aided by the presence of vectors and detection of viral DNA may not always be indicative of the presence of infectious virus (OIE 2021c).

CyHV-3 is inactivated by UV radiation (4 mWs/cm<sup>2</sup>) and temperatures above 50°C for 1 minute (Kasai, Muto & Yoshimizu 2005). CyHV-3 is also inactivated by treatment with iodophor at 200 mg/L for 20 minutes, benzalkonium chloride at 60 mg/L for 20 minutes, ethyl alcohol at 30% for 20 minutes and sodium hypochlorite at 200 mg/L for 30 seconds, all at 15°C (Kasai, Muto & Yoshimizu 2005; OIE 2021c).

## 9.2.2 Epidemiology

### Host range

In general, alloherpesviruses display a high level of host specificity and appear to cause disease in only one species of fish or in closely related members of the same genus (Hanson, Dishon & Kotler 2011).

Species which fulfil the criteria for listing as a species susceptible to infection (N= natural exposure; E= experimental exposure) with CyHV-3 in accordance with chapter 1.5 of the WOA *Aquatic animal health code* (WOAH Code) (OIE 2021a) include:

- All varieties and subspecies of *Cyprinus carpio*<sup>N, E</sup> (common carp) (Bergmann et al. 2010; Bretzinger et al. 1999; Hedrick et al. 2000; Perelberg et al. 2003; Uchii et al. 2009)
- Common carp hybrids<sup>N</sup> (e.g. *C. carpio* × *Carassius auratus*) (Bergmann et al. 2010).

Species for which there is incomplete evidence for susceptibility to infection (N= natural exposure; E= experimental exposure) according to WOAH include:

- *Carassius auratus* (goldfish)<sup>N, E</sup> (Bergmann et al. 2010; Bergmann et al. 2009; El-Matbouli & Soliman 2011)
- *Carassius carassius* (crucian carp) (Kempter et al. 2009)
- *Ctenopharyngodon idella*<sup>N</sup> (grass carp) (Bergmann et al. 2009).

CyHV-3-positive PCR results have also been reported in several species, however no active infection was demonstrated (N= natural exposure; E= experimental exposure), including:

- *Acipenser gueldenstaedtii*<sup>N</sup> (Russian sturgeon) (Kempter et al. 2009)
- *Acipenser oxyrinchus*<sup>N</sup> (Atlantic sturgeon) (Kempter et al. 2009)
- *Acipenser ruthenus* × *Huso huso*<sup>E</sup> (hybrid sterlet × beluga) (OIE 2021c; Pospichal et al. 2016)
- *Anodonta cygnea*<sup>N</sup> (swan mussel) (Kielpinski et al. 2010)
- *Barbatula barbatula* (stone loach) (OIE 2021c)
- *Gammarus pulex*<sup>N</sup> (scud, crustacean) (Kielpinski et al. 2010)
- *Gymnocephalus cernuus*<sup>N</sup> (Euraseas ruffe) (Kempter et al. 2012; OIE 2021c)
- *Hypophthalmichthys molitrix*<sup>N</sup> (silver carp) (Ilouze et al. 2011; Kempter et al. 2012)
- *Leuciscus idus*<sup>N</sup> (blue back ide) (Bergmann et al. 2009)

- *Oncorhynchus mykiss*<sup>E</sup> (Rainbow trout) (Bergmann et al. 2016)
- *Perca fluviatilis*<sup>N</sup> (European perch, redfin)(Kempton et al. 2012)
- *Rotifera* species<sup>N</sup> (Minamoto et al. 2011)
- *Rutilus rutilus*<sup>N</sup> (common roach) (Kempton et al. 2012)
- *Silurus glanis* (wels catfish) (Kempton et al. 2009)
- *Tinca tinca* (tench) (Kempton et al. 2009; OIE 2021c)
- *Vimba vimba* (vimba) (Kempton et al. 2009).

All life stages of fish, from juveniles upwards, appear to be susceptible to CyHV-3 (Bretzinger et al. 1999; Hedrick et al. 2000). Carp larvae can be resistant to CyHV-3 infection but are susceptible to infection on maturation (Ito et al. 2007).

CyHV-3 infection is considered to be specific to carp and is not considered to occur in other fish species, including sturgeon (McColl et al. 2017). It was reported that CyHV-3 can replicate in *C. auratus* (goldfish) without causing disease (El-Matbouli & Soliman 2011). However, a later study using an mRNA-specific RT-PCR developed to detect the replicating stage of CyHV-3 from fish and cultured cells (Yuasa et al. 2012) reported that *C. auratus* is not a susceptible host (Yuasa, Sano & Oseko 2013).

CyHV-3 was detected in *A. gueldenstaedtii* (Russian sturgeon) (length 25–37 cm) and *A. oxyrinchus* (Atlantic sturgeon) (length 8–12 cm) by PCR and in-situ hybridization from farms in Northern Poland (Kempton et al. 2009). All positive sturgeon samples were taken from farms also holding *C. carpio* with a previous history of KHVD outbreaks (Kempton et al. 2009). While *A. oxyrinchus* in this study showed no clinical signs, *A. gueldenstaedtii* presented some disease symptoms. However, these symptoms cannot be definitively attributed to CyHV-3 as these fish were also found to be coinfecting with white sturgeon-like iridovirus and a sturgeon herpesvirus (Kempton et al. 2009).

*Acipenser ruthenus* × *Huso huso* hybrids were found to be PCR positive for CyHV-3 following cohabitation with CyHV-3-infected *C. carpio koi* in an experimental challenge (Pospichal et al. 2016). Together, this data suggests that sturgeon may act as a vector rather than a susceptible species of CyHV-3.

There is no evidence that Australian native freshwater fish are susceptible to CyHV-3 (McColl et al. 2017). A study evaluating the susceptibility of a range of non-carp species to CyHV-3 found no clinical signs, mortalities or molecular and histological evidence consistent with CyHV-3 infection (McColl et al. 2017). The species tested included 13 native Australian fish (silver perch *Bidyanus bidyanus*, Murray cod *Maccullochella peelii*, golden perch *Macquaria ambigua*, common galaxias *Galaxias maculatus*, short-finned eel *Anguilla australis*, salmon catfish *Neoarius graeffei*, eel-tailed catfish *Tandanus tandanus*, Australian smelt *Retropinna semoni*, crimson-spotted rainbowfish *Melanotaenia duboulayi*, sea mullet *Mugil cephalus*, carp gudgeon *Hypseleotris* species, olive perchlet *Ambassis agassizii* and bony bream *Nematalosa erebi*); one introduced fish species (rainbow trout *Oncorhynchus mykiss*); a lamprey species; the crustacean *Cherax destructor*; two native amphibian species (tadpole and mature stages); two native reptilian species; chickens; and laboratory mice (McColl et al. 2017).



## Geographical distribution

CyHV-3 was first reported following detection of CyHV-3 DNA in samples taken during a mass mortality of carp in the United Kingdom (UK) in 1996 (Haenen et al. 2004). There were also early reports of infection in 1997–1998 from Germany, Israel and the United States of America (USA) (Bretzinger et al. 1999; Hedrick et al. 2000; Perelberg et al. 2003). Since then, the geographical range of CyHV-3 has become extensive, being reported in at least 33 countries from Africa, Asia, Europe and North America (Australian Government & FRDC 2022; OIE 2021c). The *Report on survey and diagnosis of fish diseases in Europe 2020* states that KHVD was reported in 9 countries including Croatia, Czech Republic, Denmark, England, Germany, Hungary, Romania, Scotland and Switzerland (EURL for Fish and Crustacean Diseases 2021).

CyHV-3 and CyHV-1 have not been recorded in Australia; however, CyHV-2 has been detected in farmed and wild *C. auratus* (goldfish) populations in Australia (Becker et al. 2014).

## Prevalence

### Sturgeon

A survey of fish farms in Poland with a known history of CyHV-3 infection in *C. carpio* or *C. carpio koi*, detected CyHV-3 by PCR in 60% (n=15) of *A. gueldenstaedtii* and in 28% (n=14) of *A. oxyrinchus* (Kempter et al. 2009).

### Carp

There are limited publications about CyHV-3 prevalence in farmed or wild populations of carp. In fishponds, morbidity of affected carp populations can be 100% (Bretzinger et al. 1999; Haenen et al. 2004). A study on the prevalence of CyHV-3 in *C. carpio* using enzyme linked immunosorbent assay antibody testing found CyHV-3 antibodies in 4% (n=82) of English and Welsh fish farms and in 37% (n=71) of 'high-risk' fisheries from within England and Wales (Taylor et al. 2010a). The study also detected CyHV-3 in 50% (n=12) of consignments of imported *C. carpio koi* from 7 countries in south-east Asia (Taylor et al. 2010a). A PCR survey for CyHV-3 in wild *C. carpio* was conducted in Lake Biwa, Japan in 2006 and 31% (n=58) of fish >300 mm tested positive (Uchii et al. 2009). Serological testing of the same fish population revealed 54% (n=61) of fish >300 mm were positive for CyHV-3 antibodies suggesting they had survived a known outbreak 2 years prior (Uchii et al. 2009). Screening of carp collected from farms in Croatia as part of a national surveillance program detected CyHV-3 at a prevalence of 6% (n=368 two-fish pools) in 2016 and 0% in 2015 and 2017 (Zrncic et al. 2020). A survey of the prevalence of CyHV-3 in wild populations of fishes in Minnesota, USA was conducted on 5 lakes in 2019 and showed 10–50% (n=730) of *C. carpio* were positive CyHV-3 whereas 0% (n=756) of 28 other native fish species tested positive (Oktener et al. 2006).

In 2020, CyHV-3 was reported in 4.9% (n=261) of farmed fish samples from Croatia and in 1.7% (n=231) of fish farms in Hungary (EURL for Fish and Crustacean Diseases 2021). Additionally, 71 outbreaks of KHVD were reported from 9 European countries in that year (EURL for Fish and Crustacean Diseases 2021).

## Mortalities

### Sturgeon

No reports were found on mortalities in farmed or wild sturgeon due to CyHV-3.

### *Carp*

In fishponds, mortality of affected carp populations can be as high as 90–100% (Bretzinger et al. 1999; Haenen et al. 2004). Mortality of up to 50% during a 10-day period were reported in *C. carpio carpio* and *C. carpio koi* farms in Israel during 1998–1999 (Hedrick et al. 2000). In 2002, a KHVD outbreak in Indonesia was associated with mortality of up to 95% in *C. carpio carpio* and *C. carpio koi* (Sunarto et al. 2011). In 2004, CyHV-3 caused a mass mortality event in wild *C. carpio* in the Chadakoin River, New York, USA, that resulted in an estimated 6,000 fish dying over several weeks (Grimmett et al. 2006). In Lake Biwa, Japan, a mass mortality of more than 100,000 wild *C. carpio* occurred in 2004 due to a CyHV-3 outbreak (Matsui et al. 2008). Mass die-offs of wild *C. carpio* occurred within lakes in south-central Ontario, Canada due to CyHV-3 in 2007 and 2008 with an estimated 12,000 and 13,000 carp dead, respectively (Garver et al. 2010). The first occurrence of CyHV-3 infection in Iraq in 2018 caused the death of millions of farmed *C. carpio* (OIE 2019). A CyHV-3 outbreak in Iowa, USA in 2022 killed thousands of young wild *C. carpio* (DNR 2022).

### **Transmission**

CyHV-3 is primarily transmitted horizontally via fish to fish contact but can also occur via faeces, urine and skin mucus secreted from infected fish (Dishon et al. 2005; Kirkland & Hick 2022; OIE 2021c; Tolo et al. 2021). Transmission experiments concluded that the potential for an infectious dose of CyHV-3 for waterborne infection in the wild is unlikely to very low, due to dilution of the virus in much larger volumes and adverse conditions for the persistence of infectious KHV (Kirkland & Hick 2022). Egg-associated transmission has not been demonstrated (OIE 2021c) but CyHV-3 has been detected in ovarian tissue (Oktener et al. 2006).

The onset of KHVD in susceptible species may take as little as 3 days through cohabitation with diseased fish but may also be prolonged up to 21 days (Bretzinger et al. 1999; Hedrick et al. 2000). The onset of disease can be influenced by factors such as water temperature, age and condition of the fish, population density and other stress factors (transportation, spawning, poor water quality) (OIE 2021c).

Plankton may be involved in the spread of KHVD. Following detection of CyHV-3 in plankton (Rotifera) that was collected from a lagoon connected to a lake where mass mortality due to KHVD was observed, it was hypothesised that CyHV-3 could adhere to plankton facilitating viral movement and transmission (Minamoto et al. 2011). Generally, carp do not feed on living plankton; however, they eat bivalves inhabiting the sediments and which are known to feed on plankton. Recently, CyHV-3 has been isolated by PCR from the intestinal contents of wild ducks implicating waterfowl and piscivorous birds as potential spreaders of the virus (Torres-Meza et al. 2020)

In general, fish that survive herpesvirus infections appear healthy and normal and can carry the herpesvirus for long periods (St-Hilaire et al. 2005; Uchii et al. 2009). For example, *C. carpio koi* that survived experimental infection with CyHV-3 were healthy and still tested positive for virus at 64 days post infection (dpi) (Gilad et al. 2004). CyHV-3 was re-isolated from surviving *C. carpio* 81 dpi after the carp were subjected to netting stress (Bergmann & Kempter 2011). Carrier fish may, when stressed, release new virus into the water and infect fish not previously exposed. Those newly infected fish may develop a disease and spread the virus to other fish; they also become carriers and may infect other fish later. It is these apparently healthy carrier fish that make it very difficult to control herpesviruses in fish (Goodwin 2012).

The introduction of CyHV-3 into new areas has primarily been attributed to the movement of live animals. In Europe, trade in live fish has been reported to increase the risk of KHVD transfer. In the UK, the first isolation of CyHV-3 was in samples from a disease outbreak in *C. carpio koi* imported from Israel in 2000. Further isolations were made at UK sites in 2000 and 2001 from *C. carpio koi* imported from the USA, Israel and Malaysia ((Way et al. 2001) cited in (Haenen et al. 2004)). CyHV-3 was restricted to ornamental carp in the UK until its isolation in 2003 from *C. carpio carpio* in the wild that was associated with large numbers of mortalities. After this event, it was suggested that the spread of CyHV-3 was linked to the rearing or holding of *C. carpio carpio*, destined for restocking fisheries, with ornamental varieties of carp (Haenen et al. 2004; Taylor et al. 2010b).

### Infectious dose

The minimum infectious dose of CyHV-3 required to cause disease in susceptible species by experimental challenge or natural infection is not known. However, CyHV-3 was experimentally transmitted to *C. carpio* (weight 10 g) by immersion of fish in 10 L tanks containing 27 PFU/mL for 40 minutes and by intraperitoneal (IP) injection (0.2 mL) with a viral inoculum of  $10^3$  PFU/mL (Perelberg et al. 2003). Infection induced terminal KHVD in about 80% of the fish (Perelberg et al. 2003). *C. carpio* (weight 90 – 140 g) were infected by immersion for 2 hours with KHV at a concentration of  $10^3$  TCID<sub>50</sub>/mL (Bergmann & Kempter 2011).

The dose of CyHV-3 required to establish infection in juvenile *C. carpio* (mean length 3 cm) through IP injection was 10 TCID<sub>50</sub>/fish and by immersion challenge ranged from 0.6–30 TCID<sub>50</sub>/mL for 2 hours (McColl & Crane 2013). In a titration experiment of CyHV-3 by IP injection, infection was established in 8/8 *C. carpio* with a dose of  $1 \times 10^3$  or  $1 \times 10^4$  TCID<sub>50</sub>/fish and 6/8 *C. carpio* with  $1 \times 10^2$  or  $1 \times 10^1$  TCID<sub>50</sub>/fish (Kirkland & Hick 2022).

*C. carpio koi* (mean weight 274 g) bath exposed for 1 hour to 12 TCID<sub>50</sub>/mL of CyHV-3 resulted in mortalities at 5, 8 and 14 dpi in koi kept at water temperatures of 28°C, 23°C and 18°C, respectively (Gilad et al. 2004). There was no mortality among fish exposed at 13°C (Gilad et al. 2004). Adult *C. carpio koi* bath exposed to isolates of CyHV-3 at  $10^{3.1}$  TCID<sub>50</sub>/mL for 2 hours or IP injected with 0.1 mL of culture medium containing  $10^3$  TCID<sub>50</sub> CyHV-3 resulted in cumulative mortalities of up to 80% and 100%, respectively, by 30 dpi (Hedrick et al. 2000).

## 9.2.3 Pathogenesis

### Tissue tropism

Gills, intestine and the skin have been suggested as major portals of entry for CyHV-3 (Costes et al. 2009; Dishon et al. 2005; Gilad et al. 2004; Ilouze, Dishon & Kotler 2006; Pikarsky et al. 2004). The virus then spreads systematically to the internal organs, primarily the gut, kidney and spleen (Costes et al. 2009). CyHV-3 has also been recovered from liver, intestine and brain of affected fish (Dishon et al. 2005; Hedrick et al. 2000).

### Tissue titre

Studies in tissues of experimentally infected *C. carpio koi* showed high DNA concentrations of CyHV-3 in the gill, kidney, spleen, liver, gut, brain and mucus with  $10^7$ – $10^9$  copies/ $10^6$  host cells (Gilad et al. 2004). *C. carpio koi* surviving infection at 62–64 dpi contained lower CyHV-3 genome copies (up to  $1.99 \times 10^2$  copies/ $10^6$  host cells) in gill, kidney or brain tissues (Gilad et al. 2004). The virus load in moribund or dead wild *C. carpio* ranged from  $6.02 \times 10^1$ – $2.39 \times 10^6$  copies/ $\mu$ g host DNA or  $5.70 \times 10^1$ – $1.19 \times 10^8$  copies/ $10^6$  host cells (Garver et al. 2010). In infected farmed *C. carpio*, CyHV-

3 ranged from 800– $1.5 \times 10^8$  copies/mg of gills, and the median concentration was  $1.46 \times 10^4$  copies/mg gills (Avarre et al. 2012).

## 9.2.4 Diagnosis

### Clinical signs

#### *Sturgeon*

No clinical signs of KHVD were reported in sturgeon that tested positive for CyHV-3 (Kempter et al. 2009). CyHV-3 has been detected in coinfections with white sturgeon-like iridovirus and a sturgeon herpesvirus (Kempter et al. 2009).

#### *Carp*

Typical clinical signs include enophthalmos (sunken eyes), pale discolouration or reddening of the skin, focal or total loss of epidermis, haemorrhages on the skin and base of the fins, over production of mucus on the skin and gills, and pale discolouration or patches on the gills (Bretzinger et al. 1999; Perelberg et al. 2003). Fish with KHVD can also display lethargy, disorientation, erratic swimming, frequent ventilation (gasping) and usually congregate around aerators where it is easier for them to take up oxygen through their damaged gills (Goodwin 2012; Haenen et al. 2004).

Secondary and concomitant bacterial and/or parasitic infections are commonly seen in diseased carp, which may affect the mortality rate and display of clinical signs of disease (Haenen et al. 2004).

Concomitant parasite infestations with *Ichthyobodo* sp., *Trichodina* sp., *Ichthyophthirius* sp., *Dactylogyrus* sp., *Chilodonella cyprini* and monogenean parasites have been detected. Secondary bacterial infections were identified with *Aeromonas* sp., *Pseudomonas* sp. and *Shewanella putrifaciens* (Haenen et al. 2004).

### Pathology

CyHV-3 can cause severe hyperplasia, lamellar fusion and adhesion of gill filaments. Foci of necrosis can occur with necrotic cells observed in the liver, gill and epidermal tissues. There is also necrosis and inflammation in the hematopoietic tissues of the kidneys, spleen and lamina propria of the intestine. Nuclear inclusions may be present in nuclei of the renal glomerular (Perelberg et al. 2003).

### Testing

Chapter 2.3.6 of the WOA *Manual of diagnostic tests for aquatic animals* (WOAH Manual) provides details of the methods currently available for targeted surveillance and diagnosis of CyHV-3 (Hedrick et al. 2000; OIE 2021c).

PCR (Bercovier et al. 2005) and real-time PCR (Gilad et al. 2004) methods described in the WOA *Manual* are recommended for presumptive and confirmatory diagnosis of CyHV-3 (OIE 2021c). Cell culture, histopathology and serology may also be used to obtain a presumptive diagnosis (OIE 2021c; St-Hilaire et al. 2005; Taylor et al. 2010a).

## 9.2.5 Treatment

There are no treatments currently available for KHVD (OIE 2021c).

## 9.2.6 Control and prevention

A safe and effective vaccine for CyHV-3 is not currently available for use in Australia. However, a live attenuated virus has been used to vaccinate carp which protects the fish from virus challenge for at

least 8 months (Ilouze et al. 2011). The effectiveness of this vaccine for sturgeon has not been investigated.

Recommended prevention measures to limit the introduction of CyHV-3 on farms include sourcing fish from disease-free stock and quarantining new fish for a period of 4 weeks to 2 months before transfer to the grow out site (OIE 2021c). Hygiene measures on site include careful handling of fish to avoid stress, safe disposal of dead fish, disinfection of eggs and regular disinfection of ponds and farm equipment (OIE 2021c).

The disinfectants effective for inactivation of CyHV-3 include iodophor at 200 mg/L, benzalkonium chloride at 60 mg/L, ethyl alcohol at 30% and sodium hypochlorite at 3 mg/L, all for 30 seconds or 20 minutes at 15°C (Kasai, Muto & Yoshimizu 2005; OIE 2021c).

### 9.2.7 Impact of the disease

CyHV-3 is a serious socio-economic threat to carp aquaculture, managed carp fisheries and the ornamental fish trade. In Israel, at the end of 1998, the year in which CyHV-3 was first reported, the loss due to disease was estimated at US\$1.2 million for the common carp industry and US\$0.8 million in ornamental carp exports (Perelberg et al. 2003). It was estimated that by 2003, CyHV-3 was costing Israel aquaculture \$3 million yearly (Perelberg et al. 2003). CyHV-3 has caused severe losses to Indonesian carp culture (both koi and common carp) with total fish mortality up to 80–95% and economic losses estimated at US\$15 million by the end of 2003 (Sunarto, Rukyani & Itami 2005). More recent reports on the economic impacts of KHV are not available.

### 9.2.8 National carp control plan

The Australian Government is investigating a long-term biological control plan to use CyHV-3 as a biological control agent for *C. carpio* to improve water quality in waterways across Australia. *C. carpio* is considered one of the worst introduced pest species in Australia that causes significant social, environmental and economic impacts. The body of evidence assembled by the national carp control plan (NCCP) supports *C. carpio* being the only susceptible species of CyHV-3. The national biomass of *C. carpio* ranges from 200,000 tonnes and possibly up to approximately 1 million tonnes under ideal breeding conditions in south-eastern Australia. Modelling for the NCCP indicates that, if successfully deployed, CyHV-3 could reduce and suppress carp populations by approximately 40–60%. However, a scenario featuring major, uncontrolled carp mortalities across a large geographic area is unlikely. Rather, NCCP research predicts that CyHV-3 is likely to produce substantial, seasonally restricted kills focused on targeted carp aggregation sites. Carcass management operations are being planned to remove the large volumes of decomposing carp expected to reduce them affecting the water quality and causing native fish kills. If CyHV-3 is eventually released as a biocontrol agent in Australia then it would likely involve 2–3 years of coordinated virus deployment (via infected carp introductions) with ongoing adaptive management beyond initial deployment (Australian Government & FRDC 2022). In this case, CyHV-3 will no longer be considered exotic to Australia and any biosecurity measures specific to CyHV-3 will be reviewed.

### 9.2.9 Current biosecurity measures

There are no biosecurity measures for live sturgeon and their reproductive material as import is not permitted.

## 9.3 Risk assessment

Based on [chapter 4](#) and the technical information about CyHV-3 presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of CyHV-3 meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 9.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for CyHV-3 were that:

- This biosecurity import risk assessment (BIRA) is generic and therefore the entry assessment assumes that CyHV-3 is present in all source countries.
- CyHV-3 is considered to be specific to carp and is not considered to replicate or cause disease in other species.
- There is one report of CyHV-3 detection in farmed sturgeon, suggesting they may act as carriers of the virus (Kempter et al. 2009). The prevalence of CyHV-3 in farmed or wild sturgeon is unknown.
- In some countries, sturgeon are reared with carp (Mihailov et al. 2020; Patriche et al. 2002). Prevalence of CyHV-3 in farmed carp can be up to 100% (Haenen et al. 2004; Sunarto et al. 2011).
- There are no reports of CyHV-3 associating with sturgeon reproductive material but disinfection of cyprinid eggs is routinely applied to control CyHV-3 infections (OIE 2021c).
- It is expected CyHV-3 can infect sturgeon life stages that would be exported to Australia.
- The viral load of CyHV-3 in infected imported live sturgeon or their reproductive material may be sufficient to cause infection in susceptible species.
- CyHV-3 only remains viable in the environment for short periods.
- Inspection of live sturgeon is unlikely to detect infection with CyHV-3 as no clinical signs of disease have been reported in sturgeon.
- Sturgeon reproductive material infected or contaminated with CyHV-3 are unlikely to be detected during inspection because there would be no clinical signs.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of CyHV-3 was estimated to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

### 9.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for CyHV-3 were that:

- CyHV-3 can be transmitted horizontally via fish to fish contact, water and contaminated fomites.
- CyHV-3 only remains viable in the environment for short periods.

- CyHV-3 could be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- CyHV-3 can infect cyprinid species present in Australia, including common carp and ornamental varieties, which are farmed. CyHV-3 has been reported to infect sturgeon but whether it causes disease in sturgeon is unclear.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are not susceptible to CyHV-3 though they may act as vectors.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the temperature range of 16–25°C for when outbreaks of CyHV-3 typically occur (Haenen et al. 2004; Hedrick et al. 2000; Perelberg et al. 2003).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infected sturgeon will be certain to be exposed to viable CyHV-3.
- Introduction of CyHV-3 into the wild may occur by direct release of imported live sturgeon or its associated wastes from the aquaculture facility into natural waters. This would be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for fish escapes or waste management that would exclude CyHV-3 from discharges.
- Wild susceptible species would be expected to be exposed to CyHV-3 released into natural waters due to its hosts being present in Australian freshwaters. Carp dominate freshwater fish communities in south-eastern Australia (Australian Government & FRDC 2022).

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure to each exposure group for CyHV-3 in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Low**.

The partial likelihood of exposure to each exposure group to CyHV-3 in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**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 CyHV-3 in **imported live sturgeon** 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:

- Farmed susceptible species—**Very low**.
- Wild susceptible species—**Very low**.

The partial annual likelihood of entry and exposure of each exposure group to CyHV-3 in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**Very low**.
- Wild susceptible species—**Very 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 CyHV-3 were that:

- CyHV-3 can be transmitted horizontally via fish to fish contact, water and contaminated. Transmission from broodstock to progeny has not been demonstrated.
- CyHV-3 only remains viable in the environment for short periods.
- It is expected that susceptible hosts in direct contact with CyHV-3-infected fish would receive an infectious dose.
- There is evidence that survivors of CyHV-3 are persistently infected with virus and may retain the virus for long periods without showing clinical signs of disease.
- CyHV-3 can infect cyprinid species present in Australia, including common carp and ornamental varieties. CyHV-3 has also been detected in sturgeon, as well as *P. fluviatilis*, *Rotifera* species, *R. rutilus*, and *T. tinca* that may act as vectors.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids do not appear to be susceptible to CyHV-3 but may act as vectors.
- Outbreaks of CyHV-3 typically occur at 16–25°C, but infections could be subclinical when water is at low temperatures (13°C).
- The likelihood of CyHV-3 establishment, following a given quantity of CyHV-3 entering the environment of an exposure group, is likely for farmed susceptible species due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- Live sturgeon or their reproductive material could be moved to other aquaculture facilities in Australia for further grow-out. CyHV-3 may establish in these facilities if present in the animals or reproductive material being translocated.
- Each state and territory have translocation protocols for aquaculture animals, which may include consideration of CyHV-3.
- If CyHV-3 were to establish on a farm it could spread to wild populations through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of CyHV-3 be suspected, and response measures initiated immediately.
- The likelihood of CyHV-3 spread from farms to wild populations via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however CyHV-3 could spread this way. CyHV-3 could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.



- Australian native species of finfish are not considered susceptible to CyHV-3 but 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 CyHV-3 were to occur in the wild, establishment and spread would be more likely than on a farm because the densities of susceptible animals would increase the opportunities for transmission. Further, the ability of fish to be subclinically infected and to remain carriers after surviving an infection aids its spread.
- The likelihood of CyHV-3 in a wild population spreading to its natural geographic limits is lower than for other hazards with wider host ranges, for example, frog virus 3.
- In the absence of effective biosecurity measures, vectors such as rotifers may be transferred into the farms from the wild through the inlet water channels.

### Conclusion

Based on these considerations and using the descriptors in Table 4, the partial likelihood of establishment and spread of CyHV-3 in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

### Determining adverse impacts resulting from the outbreak scenario

The factors considered relevant when determining the adverse impacts resulting from establishment and spread of CyHV-3 were that:

#### *Direct effects*

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

- CyHV-3 is considered to be specific to all varieties and subspecies of *C. carpio* and it is not considered to cause disease in non-cyprinid fish species. Morbidity of affected *C. carpio* populations can be 100% and mortality can be as high as 90–100%. The domestic koi carp industry, estimated to conservatively expend \$20–52 million Australia-wide (DAFF 2022), would be significantly affected by an outbreak of KHVD.
- CyHV-3 is not known to infect any native species of fish. There is not expected to be a significant impact on wild fisheries in Australia except for the commercial wild catch *C. carpio* industry.
- Based on the impact of CyHV-3 overseas, the establishment and spread of CyHV-3 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*

- CyHV-3 has a limited host range with *C. carpio* varieties and subspecies likely the only wild species affected by an outbreak.

- The national biomass of *C. carpio* ranges from 200,000 tonnes to possibly up to approximately 1 million tonnes under ideal breeding conditions. The mass mortalities caused by CyHV-3 infection in the wild carp population could result in a significant biomass that could affect water quality and cause native fish kills if not effectively removed and disposed of.
- Carp are considered one of the worst introduced pest species in Australia and there is a national carp control plan being developed to determine the feasibility of using CyHV-3 as a biological control agent for common carp in Australia (Australian Government & FRDC 2022).
- The direct impact of CyHV-3 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*

- Infection with KHV (CyHV-3) is listed as a notifiable disease by WOA and it 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 CyHV-3.
- If CyHV-3 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 CyHV-3 from wild populations is unlikely to be undertaken.
- If CyHV-3 was confirmed at a farm, then attempts at eradication would 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.
- Carp completely 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). The biomass of carp in south-eastern Australia was estimated to be 205,774 tonnes (Stuart et al. 2021). An outbreak of CyHV-3 could have up to 90% mortality, which would 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 CyHV-3 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*

- If movement control orders were put in place it would have indirect impacts on other industries such as commercial wild catch carp fisheries for human consumption and for fertiliser and bait, and the ornamental fish industry.
- CyHV-3-infected fish may show clinical signs which would affect their marketability.
- CyHV-3 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*

- Infection with KHV (CyHV-3) is a WOA-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to CyHV-3 to avoid the possible introduction of CyHV-3.
- Ornamental finfish for export may need to be tested and free of CyHV-3 as part of export certification if CyHV-3 was to become established in Australia.
- The impacts of CyHV-3 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*

- KHVD is primarily a disease of cyprinid species and would cause significant mortality in wild carp.
- There are no species listed as endangered in Australia that are known to be susceptible to CyHV-3.
- If an outbreak of CyHV-3 led to a mass fish kill and a significant biomass that was not effectively cleaned-up, it could impact local biodiversity.
- The impact of CyHV-3 establishment and spread on the biodiversity of the environment is expected to be minor at the state or territory 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 CyHV-3 are recreationally fished in Australia and could be affected by mortalities due to infection or movement restriction areas put in place which may impact on social amenity.
- Large scale mortalities may have a detrimental effect on social amenity if dead fish are not effectively removed and disposed of.
- In local areas where recreational fishing is a major industry, a KHVD outbreak could cause loss of business and welfare concerns.
- The social impacts of CyHV-3 establishment and spread are expected to be minor at the district or region level.

Table 13 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of CyHV-3. 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 13 Overall impact of establishment and spread of CyHV-3 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)	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)	District or region	Minor	C

Effects	Criteria	Level	Impact	Score
	Economic (international trade effects)	State or territory	Minor	D
	Environment (biodiversity, endangered species and the integrity of ecosystems)	State or territory	Minor	D
	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 CyHV-3 was estimated to be **moderate**.

### 9.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for CyHV-3 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

### 9.3.6 Determination of the partial annual overall risk

The partial annual risk of CyHV-3 entry, establishment and spread from **imported live sturgeon** 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:

- Farmed susceptible species—**Very low**.
- Wild susceptible species—**Very low**.

The partial annual risk of CyHV-3 entry, establishment and spread from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species—**Very low**.
- Wild susceptible species—**Very low**.

### 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 CyHV-3 was found to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

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

## 9.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the biosecurity risk for CyHV-3 in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are present here and summarised in [Appendix B](#) and [Appendix C](#).

### 9.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 14 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of CyHV-3 but which on their own do not achieve Australia's ALOP for CyHV-3 in imported live sturgeon or their reproductive material.

**Table 14 Biosecurity measures that on their own do not achieve Australia's ALOP for CyHV-3**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Disease-free stock	<b>Yes:</b> Determination of CyHV-3 freedom would need to be to a standard consistent with that recommended by the World Organisation for Animal Health (WOAH), or equivalent.
2	Post-arrival quarantine (PAQ)	<b>Yes:</b> The stress of transport could induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
3	Post-arrival batch testing	<b>Yes:</b> There are PCR and real-time PCR methods available to detect CyHV-3 (OIE 2021c). Under this scenario, testing is conducted under departmental control and oversight.

### 9.4.2 Biosecurity measures that in combination achieve Australia's ALOP

A combination of biosecurity measures 1, 2 and 3 when applied to **imported live sturgeon** would reduce the likelihood of entry of CyHV-3 from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1, 2 and 3 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of CyHV-3 from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

# 10 *Ergasilus sieboldi*

## 10.1 Background

*Ergasilus sieboldi* is an aetiological agent of ergasilosis. It is a copepod gill ectoparasite in the family *Ergasilidae*. *E. sieboldi* affects a wide range of freshwater and marine fish, including salmonids, clupeids, perches, pikes, cyprinids and catfishes (Schäperclaus 1992). *E. sieboldi* was first described by von Nordmann in 1832 and has been reported to cause mass mortalities. *E. sieboldi* has a broad geographical distribution and is found in North America, Asia, and Europe (Piasecki et al. 2004).

Infection with *E. sieboldi* is not listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (OIE 2020) and is not on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). Australia has a long history of passive surveillance and a strong system in place to detect incursions. Although some *Ergasilus* species are present in Australia, *E. sieboldi* is considered exotic to Australia.

## 10.2 Technical information

### 10.2.1 Agent properties

The life cycle of *E. sieboldi* comprises 6 nauplius stages, 5 copepodid stages and an adult stage, all of which can be free-living (Abdelhalim, Lewis & Boxshall 1991). The free-living stages feed on algae in the water (NFS 2018). Male adults die after mating, while female adults become parasitic and attach to the fish host (Abdelhalim, Lewis & Boxshall 1991). The female adults can live for approximately one year and can overwinter on fish (NFS 2018; Tildesley 2008). One female can produce approximately 200 eggs per generation with up to 3–5 generations per year. Theoretically, the ensuing second generation can therefore comprise 40,000 descendants and in the third, as many as 8 million (Schäperclaus 1992).

The life cycle of *E. sieboldi* is temperature dependant and parasite populations peak during summer (Noga 2000). Eggs start to hatch at around 8°C and at peak temperatures of 18°C, the parasite can produce and hatch a new batch of eggs every 7 days (NFS 2018). It takes around 10 weeks at 12–15°C for eggs to develop into an adult but this is reduced to 22 days in warmer conditions (NFS 2018). New infestations on fish hosts are not seen above 22°C and parasites enter an overwintering phase at temperatures below 8°C where no new eggs are produced and no new infestations initiated (Tildesley 2008). If kept in water at a temperature of <8°C, adult females can live for up to a week not attached to a host (Tildesley 2008).

*E. sieboldi* attaches to fish gills using its second antennae. The antennae, transformed into powerful hooks, hold the gill filaments tightly and the parasite feeds on mucus, blood and tissues (Johnson et al. 2004; Piasecki et al. 2004). Once attached, the parasite continues to swell and their swimming capabilities are reduced making it harder for the parasite to detach and reattach onto a new host.

### 10.2.2 Epidemiology

#### Host range

Species which are reported to be susceptible to infestation with *E. sieboldi* (N= natural exposure; E= experimental exposure) include (but are not limited to):

- *Abramis brama*<sup>N</sup> (bream) (Dezfuli et al. 2003)
- *Abramis bjoerkna*<sup>N</sup> (silver bream) (Dzika, Kuszczala & Kozłowski 2008)
- *Alburnus alburnus*<sup>N</sup> (bleak) (Dzika, Kuszczala & Kozłowski 2008)
- *Acipenser oxyrinchus*<sup>N</sup> (Atlantic sturgeon) (Popielarczyk & Kolman 2013)
- *Acipenser ruthenus*<sup>N</sup> (sterlet sturgeon) (Lieberman & Voropaeva 2018)
- *Bagrus bajad*<sup>N</sup> (bayad) (Hamouda 2018)
- *Carassius auratus*<sup>N</sup> (goldfish) (Molnar & Szekely 1995)
- *Coregonus albula*<sup>N</sup> (vendace) ((Ruotsalainen 1984) cited in (Tildesley 2008))
- *Coregonus lavaretus*<sup>N</sup> (European whitefish) (Balling & Pfeiffer 1997)
- *Coregonus peled*<sup>N</sup> (peled) ((Abrosoy & Bauer 1959, 1961) cited in (Piasecki et al. 2004))
- *Coregonus wartmanni*<sup>N</sup> (blaufelchen) ((Baumann 1913) cited in (Piasecki et al. 2004))
- *Ctenopharyngodon idella*<sup>N</sup> (grass carp) (Molnar & Szekely 1995)
- *Cyprinus carpio* (carp)<sup>N</sup> (Aydogdu et al. 2001; Molnar & Szekely 1995)
- *Esox lucius*<sup>N</sup> (pike) (Sobecka & Piasecki 2002)
- *Gobio gobio*<sup>N</sup> (gudgeon) (Dzika, Kuszczala & Kozłowski 2008)
- *Gymnocephalus cernuus*<sup>N</sup> (ruffe) (Dzika, Kuszczala & Kozłowski 2008)
- *Lepomis gibbosus*<sup>N</sup> (Pumpkinseed) ((Cakic & Hristic 1987) cited in (Djikanovic et al. 2018))
- *Mugil cephalus*<sup>N</sup> (sea mullet) (Vinobaba 2007)
- *Neogobius melanostomus*<sup>N</sup> (round goby) (Kvach 2002)
- *Oncorhynchus mykiss*<sup>N</sup> (rainbow trout) (Piasecki et al. 2004)
- *Oxyeleotris marmorata*<sup>N</sup> (marble goby) (Worananthakij & Maneepitaksanti 2021)
- *Pelecus cultratus*<sup>N</sup> (sichel) (Molnar & Szekely 1995)
- *Perca fluviatilis*<sup>N</sup> (European perch, redfin) (Tuuha, Valtonen & Taskinen 1992)
- *Rutilus rutilus*<sup>N</sup> (roach) (Suthar, Unger & Palm 2022)
- *Salmo trutta*<sup>N</sup> (brown trout) (Fryer 1969)
- *Sparus aurata*<sup>N</sup> (gilthead seabream) (Dezfuli et al. 2010)
- *Sander lucioperca*<sup>N</sup> (pike-perch) (Molnar & Szekely 1997)
- *Sander volgensis*<sup>N</sup> (Volga pikeperch) (Molnar & Szekely 1997)
- *Silurus glanis*<sup>N</sup> (Wels catfish) (Molnar & Szekely 1995)
- *Tinca tinca*<sup>N</sup> (tench) (Molnar & Szekely 1995; Schäperclaus 1992).

*E. sieboldi* infests all life stages of fish but particularly adult fish (Molnar & Szekely 1997). *E. sieboldi* infested *A. oxyrinchus* 130–178 cm in length (weight 11–74 kg) and *A. ruthenus* 27.8–51.5 cm in length (Liberman & Voropaeva 2018; Popielarczyk & Kolman 2013).

### Geographical distribution

*E. sieboldi* has a broad geographical distribution and has been reported in Austria (Dezfuli et al. 2003), Canada (Popielarczyk & Kolman 2013), Egypt (Hamouda 2018), Germany (Suthar, Unger & Palm 2022), Hungary (Molnar & Szekely 1997), Finland (Tuuha, Valtonen & Taskinen 1992), Italy (Dezfuli et al. 2010), Poland (Dzika, Kuszczala & Kozłowski 2008; Sobecka & Piasecki 2002), Republic of Türkiye (Aydogdu et al. 2001), the Russian Federation (Liberman & Voropaeva 2018), Sri Lanka (Vinobaba 2007), Switzerland (Balling & Pfeiffer 1997), Thailand (Worananthakij & Maneepitaksanti 2021) and the United Kingdom (UK) (Fryer 1969).

### Prevalence

#### Sturgeon

The prevalence of *E. sieboldi* on *A. oxyrinchus* collected from an open pond system in Poland in 2008 was 27.2% (n=11) (Popielarczyk & Kolman 2013). *A. oxyrinchus* captured from the St John River, Canada also in 2008 had a prevalence of *E. sieboldi* of 63% (n=11) (Popielarczyk & Kolman 2013). *E. sieboldi* was detected at a prevalence of 1.6% (n=63) on *A. ruthenus* sampled from the Irtys river, the Russian Federation in 2017 (Liberman & Voropaeva 2018).

#### Salmonids

In 2003–2005, samples of *O. mykiss* were taken monthly from the Rutland Water fishery, UK to monitor *E. sieboldi* presence and it was detected at a prevalence of 10–100% (Tildesley 2008). A survey of parasite fauna on *O. mykiss* from a flow-through aquaculture system at Lake Tollense, Germany in 2018 reported *E. sieboldi* in 3.1% (n=64) (Unger et al. 2022).

#### Other fish

*A. brama* captured from Lake Mondsee, Austria in 2001 were infested with *E. sieboldi* at a prevalence of 50% (n=14) (Dezfuli et al. 2003). A survey of the parasites of fish collected from Lake Kortowskie, Poland in 2001–2004 detected *E. sieboldi* at a prevalence of 83.33% on *E. lucius* (n=6), 80% on *T. tinca* (n=5), 45.45% on *G. cernuus* (n=55), 17.86% on *P. fluviatilis* (n=56), 16.66% on *A. brama* (n=12), 7.69% on *G. gobio* (n=13), 6.57% on *A. bjoerkna* (n=76), 5.12% on *R. rutilus* (n=66) and 4.76% on *A. alburnus* (n=46) (Dzika, Kuszczala & Kozłowski 2008). *M. cephalus* collected from Batticala lagoon, Sri Lanka in 2003–2004 had a prevalence of *E. sieboldi* of 31.3% (n=35) (Vinobaba 2007). In Italy, *E. sieboldi* prevalence of 74% (n=30) was reported in farmed *S. aurata* in 2009 (Dezfuli et al. 2010). *E. sieboldi* prevalence of 5% (n=120) was detected in *O. marmorata* sampled from the Bang Pakong River, Thailand during 2013–2014 (Worananthakij & Maneepitaksanti 2021). In Egypt, *B. bajad* were collected during 2017–2018 from Lake Nasser and 55% (n=100) were positive for *E. sieboldi* (Hamouda 2018). *A. brama* and *R. rutilus* sampled for parasites in 2018–2019 from Lake Tollense, Germany had a prevalence of *E. sieboldi* of 33% (n=30) and 8.5% (n=47), respectively (Suthar, Unger & Palm 2022).

### Mortalities

#### Sturgeon

No reports were found on mortalities in farmed or wild sturgeon due to *E. sieboldi*.



*Other fish*

There are limited recent publications about mortalities in wild or farmed fish due to *E. sieboldi*. It has been reported to cause mass fish-kills in *T. tinca*, *E. lucius*, *P. fluviatilis*, *O. mykiss*, *C. wartmanni* and *A. brama* (NFS 2018; Piasecki et al. 2004). In 2006, *E. sieboldi* caused 40% mortality of juvenile *S. aurata* in a semi-intensive fish farm in Italy (Dezfuli et al. 2010). In 2020, mass mortalities were observed (no numbers given) among cultured *S. aurata* on a semi-intensive marine farm in Egypt (Abdel-Radi et al. 2022).

**Transmission**

*E. sieboldi* can be transmitted via contact between fish, water or contaminated fomites (NFS 2018; Roberts 2001).

**10.2.3 Pathogenesis****Tissue tropism**

*E. sieboldi* typically attaches to gills but may also be found on the fins, body surface and in the nasal cavity (Molnar & Szekely 1997; NFS 2018; Piasecki et al. 2004).

**Tissue titre**

*E. sieboldi* has been recorded from farmed *A. oxyrinchus* in Poland at 0–1 parasites per fish (Popielarczyk & Kolman 2013). *E. sieboldi* was observed on the skin of *A. oxyrinchus* from St John River in Canada at 1–18 parasites per fish (Popielarczyk & Kolman 2013).

An infested *T. tinca* was reported having 3600 specimens of *E. sieboldi* on its gills (Schäperclaus 1992). A single *S. trutta* collected from a reservoir in the UK carried over 130 *E. sieboldi* on its gill filaments (Fryer 1969). The number of parasites on *P. fluviatilis* sampled from lakes in Finland ranged from 2–10 (Tuuha, Valtonen & Taskinen 1992). The gills of *A. brama* from Lake Mondsee, Austria, were parasitized with *E. sieboldi* at an intensity of 1–23 parasites per fish (Dezfuli et al. 2003). *E. sieboldi* present on the gills of farmed *S. aurata* ranged from 3–50 parasites per fish (Dezfuli et al. 2010). *M. cephalus* captured from Batticala lagoon, Sri Lanka were infested with *E. sieboldi* at a range of 1–6 parasites per fish (Vinobaba 2007). The number of *E. sieboldi* on wild *S. lucioperca* ranged from 7–800 on the operculum and 2–80 on the gills (Molnar & Szekely 1997).

**10.2.4 Diagnosis****Clinical signs**

Severity of the disease is dependent on the number of *Ergasilus* infesting the fish as well as on the size and age of the fish, the health of the fish and the developmental stages of *Ergasilus* present (Johnson et al. 2004). When present in low numbers, *Ergasilus* cause relatively minor effects to the host (Kabata 1988; Schäperclaus 1992). Heavy infestations can result in a loss of fitness, slow growth, lethargy, abnormal swimming and mortality (NFS 2018; Piasecki et al. 2004). Excess mucus secretion has also been observed (Dezfuli et al. 2003; Dezfuli et al. 2010).

**Pathology**

The attachment of *E. sieboldi* to the gills of fish can cause tissue damage, necrosis, haemorrhage and hyperplasia (Molnar & Szekely 1997). Heavy infestations will reduce the surface area and function of the gills which causes respiratory distress and loss of osmoregulatory function (Molnar & Szekely 1997; NFS 2018). The damage to the gills can often lead to secondary bacterial and fungal infections (Dezfuli et al. 2003; Fryer 1969).

### Testing/detection

Adult parasites are approximately 1.7 mm long and can be readily detected with the naked eye and diagnosis is made by morphological identification (Kabata 1988; Noga 2000).

### 10.2.5 Treatment

Chemicals used for the treatment of other crustacean ectoparasites, such as organophosphates or salt, can be effective against *E. sieboldi* (Noga 2000). However, like for argulosis, chemical resistance, availability, legality of use on food fish species, fish species sensitivity, impact on the environment, dosage rates and costs must be considered (Hakalahti-Sirén, Mikheev & Valtonen 2008; Kumar et al. 2017; Steckler & Yanong 2012). Repeated treatments may be required if the chemical is only effective against one life cycle stage to ensure all parasites are eradicated.

### 10.2.6 Control and prevention

Management practices to control an infestation in aquaculture facilities include limiting the movement of fish from infested waters, only stocking with *E. sieboldi*-negative fish, maintaining low stocking densities, ensuring good water quality and fallowing and drying sites between successive stockings (Jithendran, Natarajan & Azad 2008; NFS 2018).

### 10.2.7 Impact of the disease

*E. sieboldi* causes serious disease problems in fisheries with losses the result of direct mortality, mortality due to secondary infections, reduced growth and costs associated with treatment (Johnson et al. 2004; Schäperclaus 1992). In Lake Scharmuzel, Germany, the yield of *T. tinca* in a farm dropped from 5000 kg before *E. sieboldi* became established to 350 kg after its introduction (Piasecki et al. 2004). In 2 other German lakes, the yield of *T. tinca* dropped from 31–47 kg/ha to 16.5 kg/ha after the invasion of *E. sieboldi* (Piasecki et al. 2004). Production of farmed *S. aurata* suffered annual losses of approximately 5–8% of the juvenile stock due to *E. sieboldi* (Dezfuli et al. 2010).

### 10.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon or their reproductive material as import is not permitted.

There are biosecurity measures to manage the risk of *E. sieboldi* on imported ornamental fish for display purposes (see [Appendix D](#)).

## 10.3 Risk assessment

Based on [chapter 4](#) and the technical information about *E. sieboldi* presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of *E. sieboldi* meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#).

### 10.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that *E. sieboldi* is present in all source countries.
- *E. sieboldi* infests live sturgeon that would be of a life stage exported to Australia.

- There was one report of *E. sieboldi* in farmed sturgeon in Poland at a prevalence of 27% (Popielarczyk & Kolman 2013). Prevalence in wild sturgeon ranges from 1.6–63% (Liberman & Voropaeva 2018; Popielarczyk & Kolman 2013).
- Prevalence of *E. sieboldi* in other farmed fish can reach 100% and in wild populations can be up to 83%.
- Sturgeon reproductive material is not expected to be infested with the parasites as *E. sieboldi* attach and feed on fish after they hatch.
- All life cycle stages of the parasite can survive independently of the fish host.
- Adult ectoparasites are likely to be detected on live sturgeon during inspection. However, low-level infestations, infestations of young ectoparasites and fish showing mild or no clinical signs would be unlikely to be detected.
- *E. sieboldi* have a high reproductive rate that can result in rapid escalation of infestations.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of *E. sieboldi* was estimated to be:

- Imported live sturgeon—**Moderate**.
- Imported sturgeon reproductive material—**Negligible**.

Therefore, only live sturgeon were considered further in the risk assessment.

### 10.3.2 Exposure assessment

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

- *E. sieboldi* can be transmitted via contact between fish, water and contaminating fomites.
- Any viable *E. sieboldi* which enter the environment would be capable of persisting as free-living parasite for a period.
- *E. sieboldi* have a high reproductive rate that can result in rapid escalation of infestations.
- Species susceptible to *E. sieboldi* infestation are present in Australia including *C. carpio*, *M. cephalus*, *P. fluviatilis*, *R. rutilus*, *T. tinca* and salmonids.
- Aquaculture species most likely to be polycultured with imported sturgeon such as trout are susceptible to *E. sieboldi*.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the temperature range for *E. sieboldi* reproduction (Noga 2000).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infested sturgeon will be certain to be exposed to viable *E. sieboldi*.
- Introduction into the wild may occur by direct release of imported live sturgeon or its associated wastes from the aquaculture facility into natural waters. This would be a direct pathway to wild

susceptible species if a farm has not implemented standards of biosecurity for fish escapes and waste management that would exclude *E. sieboldi* from discharges.

- Wild susceptible species would be less abundant than susceptible species in aquaculture facilities. Despite this, wild susceptible species would be expected to be exposed *E. sieboldi* released into natural waters due to its ability to survive independently from the host, and its wide host range present in Australian waters.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to *E. sieboldi* on **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Moderate**.

### ■ Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to *E. sieboldi* on **imported live sturgeon** 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

## 10.3.3 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. sieboldi* were that:

- *E. sieboldi* can be transmitted via contact between fish, water and contaminating fomites. It can also survive as a free-living parasite in the aquatic environment.
- *E. sieboldi* adult females survive on fish for up to a year.
- Aquaculture and wild fish species present in Australia are susceptible to *E. sieboldi*, including *C. carpio*, *M. cephalus*, *P. fluviatilis*, *R. rutilus*, *T. tinca* and salmonids.
- The likelihood of *E. sieboldi* establishment, following a given quantity of *E. sieboldi* entering the environment of an exposure group, is high for farmed susceptible species. This is due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- If infested live sturgeon or polycultured susceptible species in an aquaculture facility were moved to another aquaculture facility in Australia, it is likely that *E. sieboldi* would establish in these facilities.
- Each state and territory have translocation protocols for aquaculture animals but some disease agents may not be covered, including *E. sieboldi*.
- There are treatment options for *Ergasilus* infestations but repeated treatments and careful chemical selection, if available in Australia, is required.

- If *E. sieboldi* were to establish on a farm it could spread to neighbouring farms and wild populations through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of *E. sieboldi* be suspected and response measures initiated immediately. However, *E. sieboldi* is effectively transmitted through water and can persist in the environment as a free-living parasite, and farms which share a common water source with an infected population may be exposed to *E. sieboldi*.
- The likelihood of *E. sieboldi* spread from farms to wild populations or neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however *E. sieboldi* could spread this way. *E. sieboldi* could also be spread from farms to wild populations via birds scavenging infested moribund fish and dropping them into unaffected waters.
- If *E. sieboldi* were to establish in salmonid aquaculture facilities then the summer water temperatures where salmonids are farmed would favour the spread of *E. sieboldi*.
- Spread of *E. sieboldi* from farmed to wild susceptible species may occur through the movement of infested polyculture species (e.g. rainbow trout, brown trout and Chinook salmon) into natural waters to replenish depleted populations. *E. sieboldi* could be effectively transferred this way because fish may not show obvious infestation or clinical signs before transfer.
- If one or more index cases of *E. sieboldi* were to occur in the wild, establishment and spread would be similar to on a farm because the wide host range of susceptible animals increases opportunities for transmission. Further, because *E. sieboldi* can survive in the environment as a free-living parasite for a period, it could persist until susceptible hosts were to encounter it.
- The likelihood of *E. sieboldi* in a wild population spreading to its natural geographic limits is greater than for other hazards with moderate host ranges, such as typical *Aeromonas salmonicida*, and would be more likely than for those hazards which cannot survive outside of a host for long periods. The ability of fish to be unaffected by low levels of infestation and the parasite's ability to survive on fish for long periods also aids its spread.
- If *E. sieboldi* were to establish in the wild, especially in waters around aquaculture facilities, it may easily spread to farms through water intake due to *E. sieboldi* being able to survive as a free-living parasite and being transmissible through water. In the absence of effective biosecurity measures, wild infested fish may be transferred into the farms through the inlet water channels.

## Conclusion

Based on these considerations and using the descriptors in Table 4, the partial likelihood of establishment and spread of *E. sieboldi* in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**High**.

## Determining adverse impacts resulting from the outbreak scenario

The factors considered relevant when determining the adverse impacts resulting from establishment and spread of *E. sieboldi* were that:

### *Direct effects*

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

- Fish farmed in Australia are susceptible to *E. sieboldi*. There is high morbidity and mortality associated with heavy infestation.
- Production losses in a sturgeon aquaculture industry would be significant considering sturgeon require >3 years to reach sexual maturity.
- Production and productivity losses due to *E. sieboldi* would also be significant for the Australian salmonid industry with aquaculture production valued at approximately AU\$1 billion in 2020–21 (Tuynman & Dylewski 2022).
- *E. sieboldi* has been detected in wild fish populations but there are no reports of associated declines in catch rates or mortalities.
- Based on the impacts of *E. sieboldi* infestation in fish farming industries overseas, *E. sieboldi* 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*

- Susceptible species are distributed in Australian waters and *E. sieboldi* has been detected in wild fish populations elsewhere in the world. However, there have been no reports of mortalities in the wild due to *E. sieboldi*.
- The direct impact of *E. sieboldi* 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*

- Infestation with *E. sieboldi* is not listed as a notifiable disease by WOAHA nor is it on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Although not listed in Australia, state and territory governments would be expected to report on the presence of *E. sieboldi*.
- If *E. sieboldi* was confirmed at a farm, then attempts at eradication would likely be undertaken. Ergasilosis can be treated with chemicals and controlled by various management practices. However, repeated treatments would be required to remove all the ectoparasites and its free-living stages and the chemicals must be approved for use in Australia and aquaculture.
- 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 eradication was unsuccessful, then on-going control programs may need to be developed to control outbreaks of *E. sieboldi* in aquaculture facilities.
- If a movement restricted area were put in place for an infestation of *E. sieboldi*, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- If *E. sieboldi* was confirmed in the environment, eradication would be near impossible as all parasite stages can survive independently of the fish host.

- Eradication and control of *E. sieboldi* 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 areas put in place to control an outbreak of *E. sieboldi* would have indirect impacts on other industries such as seafood suppliers and ornamental fish facilities due to the host range of *E. sieboldi*.
- 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.
- *E. sieboldi*-infested fish may show gross signs which may affect their marketability.
- *E. sieboldi* 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 *E. sieboldi* is not a WOAHL-listed disease. Importing countries may have import requirements or have closed borders to the import of live species susceptible to *E. sieboldi* to avoid the possible introduction of *E. sieboldi*.
- If *E. sieboldi* were to become established, Australia could use zoning to maintain or gain access to international markets for live fish and, if required, non-viable product.
- The impacts of *E. sieboldi* establishment and spread on international trade is not expected to be discernible at any level.

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

- *E. sieboldi* has a wide susceptible host range but is not considered to cause significant mortality in wild susceptible finfish.
- No endangered Australian finfish species are currently known to be susceptible to infestation with *E. sieboldi*.
- The impact of *E. sieboldi* establishment and spread on the biodiversity of the environment 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 *E. sieboldi* are recreationally fished in Australia and could be affected by movement restriction areas put in place due to an outbreak of *E. sieboldi* which may impact on social amenity. This includes impacts on important species for indigenous cultural fishing, such as perch, snapper and bream.
- Fish infested with *E. sieboldi* also deters recreational fishers because the appearance of the fish is unappealing.



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

Table 15 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of *E. sieboldi*. 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 15 Overall impact of establishment and spread of *E. sieboldi* 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)	Local	Minor	B
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)	Local	Unlikely to be discernible	A
	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)	District or region	Minor	C

### Conclusion

The overall impact of establishment and spread of *E. sieboldi* was estimated to be **moderate**.

### 10.3.4 Determination of likely consequences of the outbreak scenario

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

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

### 10.3.5 Determination of the partial annual risk

The partial annual risk of *E. sieboldi* entry, establishment and spread 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.



### 10.3.6 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 risk associated with *E. sieboldi* was found to be:

- Imported live sturgeon—**Moderate**.

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

## 10.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for *E. sieboldi* in imported live sturgeon to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#).

### 10.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 16 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of *E. sieboldi* but which on their own do not achieve Australia's ALOP for *E. sieboldi* in imported live sturgeon.

**Table 16 Biosecurity measures that on their own do not achieve Australia's ALOP for *E. sieboldi***

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Disease-free stock	<b>Yes:</b> Determination of <i>E. sieboldi</i> freedom would need to be to a standard consistent with that recommended for World Organisation for Animal Health (WOAH) listed diseases, or equivalent.
2	Pre-export parasite treatment	<b>Yes:</b> <i>E. sieboldi</i> are sensitive to parasite treatment. However, treatments can be ineffective at removing all the parasites so reinfestation can occur.
3	Post-arrival quarantine (PAQ)	<b>Yes:</b> Infested sturgeon can be detected during the PAQ period.
4	Post-arrival parasite treatment	<b>Yes:</b> <i>E. sieboldi</i> are sensitive to parasite treatment. Treatments may need to be repeated to be effective.

### 10.4.2 Biosecurity measures that in combination achieve Australia's ALOP

Either biosecurity measure 1 or 2, in combination with 3 and 4 when applied to **imported live sturgeon** would reduce the likelihood of entry of *E. sieboldi* from **moderate** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

# 11 Frog virus 3

## 11.1 Background

Frog virus 3 (FV3) infects and causes disease in fish, amphibians and reptiles (Chinchar, Waltzek & Subramaniam 2017). FV3 is a species of the genus *Ranavirus* in the family *Iridoviridae* (Chinchar & Waltzek 2014). FV3 has a broad host range and the potential to cause considerable morbidity and mortality in both captive and wild animals (Chinchar, Waltzek & Subramaniam 2017; Duffus et al. 2015).

FV3 was first isolated from *Lithobates pipiens* (northern leopard frog) in the United States of America (USA) in the 1960s (Granoff, Came & Breeze 1966). Cases of ranavirus infection have since been documented on 6 continents and in at least 175 species of ectothermic vertebrates (Duffus et al. 2015). FV3 has the widest known geographic range of all ranaviruses and outbreaks have occurred in North America, South America, Asia and Europe (Chinchar & Waltzek 2014; Duffus et al. 2015).

Infection with ranavirus (including FV3) is listed as a disease notifiable to the World Organisation for Animal Health (WOAH) for amphibians (OIE 2020). However, FV3 detected in fish or reptiles is not reportable. Similarly, infection of amphibians with ranavirus is on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a).

There is significant uncertainty in the taxonomy of isolates within the genus ranavirus and the species FV3. 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). Several isolates of FV3, often termed FV3-like viruses, have been identified including German gecko ranavirus (GGRV), rana gyrlio virus (RGV), soft-shelled turtle iridovirus (STIV), tadpole virus 3 (TV2), tortoise virus 5 (TV5) and tiger frog virus (TFV) (Chinchar, Waltzek & Subramaniam 2017; OIE 2021d; Price 2015). In some studies, Bohle iridovirus (BIV) is classified as a FV3-like virus (Chinchar et al. 2017; Maclaine et al. 2020; Price et al. 2017; Vilaca et al. 2019) whereas in others it is not (Hyatt et al. 2000; Owen 2022). BIV is present in amphibians and reptiles in Australia (Maclaine et al. 2020; Speare & Smith 1992). However, until the status of isolates classified within the FV3-like viruses is clarified and given that ranaviruses from different geographic regions are distinguishable (Hyatt et al. 2000), a conservative approach has been taken and FV3 is still considered exotic to Australia.

## 11.2 Technical information

### 11.2.1 Agent properties

FV3 is a large (165–170 nm), icosahedral, double-stranded DNA virus that can be found in an enveloped or non-enveloped form (Chinchar et al. 2017; OIE 2021d). FV3 is classified by the International Committee on Taxonomy of Viruses (ICTV) as a member of the genus *Ranavirus* in the subfamily *Alphairidovirinae* and family *Iridoviridae* (Chinchar et al. 2017). The principal component of the viral capsid is the major capsid protein which has historically been used to distinguish FV3 and FV3-like viruses from other species of *Ranavirus* (Chinchar et al. 2017; Mao et al. 1996).

There is some evidence that FV3-like viruses from aquaculture facilities have greater virulence compared to those found in the wild (Hoverman et al. 2011; Hoverman, Gray & Miller 2010; Majji et

al. 2006; Miller, Gray & Storfer 2011). For example, experimental challenge studies using a FV3-like virus from an infected *Lithobates catesbeianus* (American bullfrog) in an aquaculture facility caused greater mortality in susceptible amphibian species compared to FV3 from a wild *Lithobates pipiens* (northern leopard frog) (Hoverman et al. 2011; Hoverman, Gray & Miller 2010).

FV3 can survive outside its hosts but survivability is temperature dependent (Gray, Miller & Hoverman 2009; Miller, Gray & Storfer 2011). Under experimental conditions, the time required for a 90% reduction in the FV3 titre (T-90 value) at 20°C was 22 days in sterile and unsterile pond water (Nazir, Spengler & Marschang 2012). The T-90 values at 4°C were 120 days in sterile pond water, 62 days in unsterile pond water and 33 days in soil (Nazir, Spengler & Marschang 2012). A second study by Munro et al. (2016) reported T-90 values of 5 days at 30°C and 34 days at 4°C in untreated lake water (Munro et al. 2016). In sediment, the T-90 values were 1 day at 30°C and 10 days at 4°C (Munro et al. 2016). Johnson and Brunner (2014) found the T-90 value for a FV3-like ranavirus at 22–24°C in filter-sterilised water was 8 days and 1 day in unsterile pond water (Johnson & Brunner 2014). Together, these results suggest that FV3 would not remain infectious outside of the host for long at high temperatures but in temperate locations during winter, sediment or water could retain sufficient virus to remain infectious weeks after contamination (Munro et al. 2016).

FV3 survives freezing as frozen FV3 stocks used in challenge studies induced infection in healthy host animals (Chen, Zheng & Jiang 1999; Hoverman et al. 2011; Schock et al. 2008; Waltzek et al. 2014; Zhang et al. 2001). FV3-like viruses can be partially inactivated by heat treatment at 56°C for 30 minutes and are sensitive to pH 3, pH 10, chloroform, 5-iodo-2-deoxyuridine, chlorhexidine, sodium hypochlorite and potassium peroxydisulfate (Bryan et al. 2009; Chen, Zheng & Jiang 1999; Prasankok, Chutmongkonkul & Kanchankhan 2005).

### 11.2.2 Epidemiology

#### Host range

Species which are reported to be susceptible to infection (N=natural exposure; E=experimental exposure) with FV3 or FV3-like viruses include but are not limited to:

#### Fish

- *Acipenser fulvescens*<sup>E</sup> (lake sturgeon) ((N. Heil & R. Bakal pers. comm.) cited in (Waltzek et al. 2014))
- *Acipenser gueldenstaedtii*<sup>N, E</sup> (Russian sturgeon) (Waltzek et al. 2014)
- *Acipenser schrenckii* (Amur sturgeon) (Duffus et al. 2015)
- *Acipenser transmontanus*<sup>N</sup> (white sturgeon) (Waltzek et al. 2014)
- *Amerius melas*<sup>E</sup> (black bullhead) (Gobbo et al. 2010)
- *Esox lucius*<sup>E</sup> (Northern pike) (Bang Jensen, Kjær Ersbøll & Ariel 2009)
- *Gambusia affinis*<sup>E</sup> (mosquito fish) (Brenes et al. 2014a; Brenes et al. 2014b)
- *Gasterosteus aculeatus*<sup>N</sup> (three spine stickleback) (Mao et al. 1999)
- *Ictalurus punctatus*<sup>E</sup> (channel catfish) (Brenes et al. 2014b)
- *Oxyeleotris marmorata*<sup>N</sup> (marble goby) (Prasankok, Chutmongkonkul & Kanchankhan 2005)

- *Sander lucioperca*<sup>E</sup> (pike-perch) (Bang Jensen et al. 2011)
- *Scaphirhynchus albus*<sup>N, E</sup> (pallid sturgeon) (Waltzek et al. 2014).

#### Amphibians

- *Ambystoma maculatum*<sup>N</sup> (spotted salamander) (Docherty et al. 2003)
- *Atelognathus patagonicus*<sup>N</sup> (Patagonia frog) (Fox et al. 2006)
- *Bufo bufo*<sup>N</sup> (common toad) (Cunningham et al. 2007)
- *Hoplobatrachus tigerinus*<sup>N</sup> (Indian bullfrog) ((Kanchanakhan 1998) cited in (Miller, Gray & Storfer 2011))
- *Hyla chrysoscelis*<sup>N, E</sup> (Cope's gray treefrog) (Brenes et al. 2014a; Hoverman, Gray & Miller 2010; Hoverman et al. 2012)
- *Hynobius nebulosus*<sup>N</sup> (Mitsjama salamander) (Une et al. 2009a)
- *Lithobates capito*<sup>N</sup> (gopher frog) (Hartmann et al. 2022)
- *Lithobates catesbeianus*<sup>N</sup> (American bullfrog) (Majji et al. 2006; Miller et al. 2007)
- *Lithobates clamitans*<sup>N</sup> (green frog) (Hoverman et al. 2012)
- *Lithobates grylio*<sup>N, E</sup> (pig frog) (Zhang et al. 2001)
- *Lithobates palustris*<sup>N, E</sup> (pickerel frog) (Hoverman, Gray & Miller 2010)
- *Lithobates pipiens*<sup>N, E</sup> (Northern leopard frog) (Granoff, Came & Breeze 1966; Schock et al. 2008)
- *Lithobates sphenoccephalus*<sup>N</sup> (Southern leopard frog) (Hoverman et al. 2012)
- *Lithobates sylvaticus*<sup>N, E</sup> (wood frog) (Duffus et al. 2008; Harp & Petranksa 2006; Schock et al. 2008)
- *Neurergus crocatus*<sup>N</sup> (spotted newt) (Stohr et al. 2013)
- *Notophthalmus perstriatus*<sup>N</sup> (striped newt) (Hartmann et al. 2022)
- *Pelophylax esculentus*<sup>N</sup> (edible frog) (Ariel et al. 2009)
- *Pseudacris regilla*<sup>E</sup> (Pacific tree frog) (Schock et al. 2008)
- *Rana aurora*<sup>N</sup> (Northern red-legged frog) (Mao et al. 1999)
- *Rana dybowskii*<sup>N</sup> (Dybowski's frog) (Xu et al. 2010)
- *Rana latastei*<sup>E</sup> (Italian agile frog) (Pearman et al. 2004; Pearman & Garner 2005)
- *Rana temporaria*<sup>N</sup> (common frog) (Cunningham et al. 1996)
- *Xenopus laevis*<sup>N</sup> (African clawed frog) (Robert et al. 2007; Soto-Azat et al. 2016).

#### Reptiles

- *Anolis carolinensis*<sup>N</sup> (green anole) (Stohr et al. 2013)
- *Anolis sagrei*<sup>N</sup> (brown anole) (Stohr et al. 2013)
- *Apalone ferox*<sup>E</sup> (Florida softshell turtle) (Brenes et al. 2014b)

- *Chelydra serpentina*<sup>N</sup> (common snapping turtle) (Carstairs, Kyle & Vilaça 2020; McKenzie et al. 2019)
- *Chrysemys picta*<sup>N</sup> (painted turtle) (Carstairs, Kyle & Vilaça 2020)
- *Geochelone platynota*<sup>N</sup> (Burmese star tortoise) (Johnson et al. 2008)
- *Graptemys pseudogeographica*<sup>E</sup> (false map turtle) (Brenes et al. 2014b)
- *Japalura splendida*<sup>N</sup> (green striped tree dragon) (Behncke et al. 2013)
- *Morelia viridis*<sup>N</sup> (green python) (Hyatt et al. 2002)
- *Pelodiscus sinensis*<sup>N</sup> (Chinese softshell turtle) (Chen, Zheng & Jiang 1999; Huang et al. 2009)
- *Pogona vitticeps*<sup>N</sup> (central bearded dragon) (Stohr et al. 2013)
- *Terrapene carolina*<sup>N</sup> (eastern box turtle) (Allender et al. 2011; Mao, Hedrick & Chinchar 1997)
- *Terrapene ornata*<sup>E</sup> (ornate box turtle) (Johnson, Pessier & Jacobson 2007)
- *Testudo horsfieldii*<sup>N</sup> (Russian tortoise) (Mao, Hedrick & Chinchar 1997)
- *Trachemys scripta elegans*<sup>E</sup> (red-eared slider turtle) (Brenes et al. 2014a; Johnson, Pessier & Jacobson 2007)
- *Trioceros melleri*<sup>N</sup> (Meller's chameleon) (Peiffer et al. 2019).

FV3-positive PCR results have been reported in a few fish species (N= natural exposure; E= experimental exposure), however no active infection has been demonstrated, including:

- *Gnathopogon species*<sup>N</sup> (cyprinid fish) (Une et al. 2009b)
- *Pimephales promelas*<sup>N</sup> (fathead minnow) (Waltzek et al. 2014)
- *Sander vitreus*<sup>N</sup> (walleye) (Waltzek et al. 2014).

The susceptibility of host species varies among fish, amphibian and reptile species and developmental stages (Hoverman, Gray & Miller 2010; Miller, Gray & Storfer 2011; Schock et al. 2008). FV3 infects juvenile sturgeon (Waltzek et al. 2014).

In amphibians, FV3 infects all life stages including larvae, metamorphs and adults (OIE 2021d). Although, most infections in otherwise healthy adult frogs are readily resolved and mortality is minimal whereas larvae and immunocompromised adults experience considerable morbidity and mortality (Robert et al. 2005; Tweedell & Granoff 1968).

Hatchling, juvenile and adult chelonians are affected by FV3 with infection often acute and fatal (Allender et al. 2011; Allender et al. 2013a; Allender et al. 2013b; Brenes et al. 2014b; Duffus et al. 2015; Johnson, Pessier & Jacobson 2007).

### Geographical distribution

FV3 and FV3-like viruses have been reported in Argentina (Fox et al. 2006), Canada (Duffus et al. 2008; Schock et al. 2008), Chile (Soto-Azat et al. 2016), China (Chen, Zheng & Jiang 1999; Zhang et al. 2001), Costa Rica (Whitfield et al. 2013), Denmark (Ariel et al. 2009), Iraq (Stohr et al. 2013), Japan (Une et al. 2009b), Thailand ((Kanchanakhon 1998) cited in (Miller, Gray & Storfer 2011))(Sriwanayos

et al. 2020), United Kingdom (UK) (Cunningham et al. 1996) and USA (Granoff, Came & Breeze 1966; Mao et al. 1999; Waltzek et al. 2014).

### Prevalence

Data on the prevalence of FV3 and FV3-like infections is low, likely due to the high mortality with quick progression to death associated with the disease (Allender et al. 2013a; Allender et al. 2013b; Gray & Miller 2013).

#### Fish

No reports of the prevalence of FV3 in fish, including in farmed or wild sturgeon, were found.

#### Amphibians

FV3 was detected in 34% (n=104) of *L. catesbeianus* and 30% (n=80) of *L. clamitans* tadpoles inhabiting 8 farm ponds in Tennessee, USA in 2005 (Gray et al. 2007). Duffus et al. (2008) found 0–80% of wild *L. sylvatica* tadpoles collected from 3 ponds in Ontario, Canada in May 2005 were positive for FV3 whereas 0% from the same ponds were positive in June 2005 (Duffus et al. 2008). Wild *R. dybowskii* collected from 7 sites in the Heilongjiang Province, China had a FV3 prevalence of 5.7% (n=315) in adults and 42.5% (n=120) in tadpoles (Xu et al. 2010). FV3 prevalence in wild *X. laevis* adults collected from 7 sites in Chile in 2011–2013 was 4% (n=175) (Soto-Azat et al. 2016). Wild *L. sylvatica* adults were collected from 28 ponds in Alabama through to Maine, USA and into Nova Scotia, Canada during 2011–2012 and had a FV3 prevalence of 38.9% (n=753) (Crespi et al. 2015). Surveys of adult, Costa Rican amphibians reported a ranavirus (species not specified) prevalence of 16.6% (n=253) across 9 amphibian species (Whitfield et al. 2013).

#### Reptiles

Wild *T. carolina carolina* collected in Tennessee, USA in 2007 had a FV3 prevalence of 1.4% (n=140) (Allender et al. 2011). A survey of wild *T. carolina carolina* sampled from Tennessee, Virginia, North Carolina, Alabama and Georgia, USA from 2007–2011 detected FV3 at a prevalence of 1.5% (n=532) (Allender et al. 2013a). *C. picta* and *C. serpentina* that had succumbed to traumatic injuries were collected in Ontario, Canada in 2014–2018 and were positive for FV3 at a prevalence of 15% (n=46) (Carstairs, Kyle & Vilaça 2020).

No reports of the prevalence of FV3 in lizards or snakes were found.

### Mortalities

Ranavirus disease typically emerges rapidly with mortality exceeding 90% in multiple species within several days (Gray & Miller 2013; Green, Converse & Schrader 2002). Mortality up to 100% has been reported for FV3 in natural and experimental infections (Chinchar, Waltzek & Subramaniam 2017; Cunningham et al. 1996; Harp & Petranksa 2006; Pearman et al. 2004; Zhang et al. 2001). In captivity, 100% mortality of hosts is commonly observed likely due to abundant hosts, guaranteed transmission and stress associated with these environments (Waltzek et al. 2014).

#### Fish

In 2001 and 2009, juvenile *S. albus* reared at a hatchery in Missouri, USA experienced 95–100% cumulative mortality due to FV3 outbreaks (Waltzek et al. 2014). Heavy mortalities (up to 90–100%) are also reported to have occurred in the same *S. albus* hatchery in 2013 and 2015 ((Stilwell 2017) cited in (Stilwell et al. 2022)). FV3 was isolated from dying juvenile *A. gueldenstaedtii* (no numbers given) at a hatchery in Georgia, USA, in 2005 with the virus isolate later proved lethal by injection in

both juvenile *A. gueldenstaedtii* and *A. fulvescens* (N. Heil & R. Bakal pers. comm. cited in (Waltzek et al. 2014)). An unusual mortality event (no numbers given) also occurred in juvenile *A. transmontanus* on a farm in California, USA, in 1998 and a FV3-like virus was detected (Waltzek et al. 2014).

### Amphibians

*L. sylvatica* tadpoles collected in 2000 near Little Bear Lake, Canada and moved to mesocosms mostly (hundreds) died within 2 weeks (Schock et al. 2008). In 2006, a commercial *L. catesbeianus* ranaculture facility in the USA suffered >50% mortality of frogs that had recently undergone metamorphosis due to FV3 (Miller et al. 2007). A mass die-off of wild *L. catesbeianus* larvae was discovered in a pond in Japan in 2008, during which several thousand carcasses were collected daily (Une et al. 2009b). Populations of *R. temporaria* in the UK that experienced reoccurring die-offs from ranaviral disease, likely FV3, declined by 81% between 1996–2008 (Teacher, Cunningham & Garner 2010). A mass die-off of >200,000 wild *L. sylvaticus* tadpoles occurred within 24 hours in a pond in Maine, USA in 2013 and was attributed to FV3 (Wheelwright et al. 2014). A persistent 2-month long outbreak of FV3-like virus in a natural community of amphibians in Florida, USA in 2021 led to a mass die-off of *L. capito* tadpoles (Hartmann et al. 2022).

### Reptiles

*T. carolina carolina* in a sanctuary in Pennsylvania, USA suffered a FV3 outbreak in 2003 and 23% of the turtles died (Johnson et al. 2008). There was 71.6% mortality of *T. carolina carolina* collected from the wild in Indiana, USA in 2010–2014 before intended relocation due to FV3 (Kimble et al. 2017). Three FV3 outbreaks occurred across 2 *T. carolina carolina* populations in Illinois, USA in 2013–2015 resulting in mortalities (numbers not provided) (Adamovicz et al. 2018). Juvenile *T. melleri* housed at Maryland Zoo, USA suffered 100% mortality over a period of 1 month due to FV3 infection (Peiffer et al. 2019).

### Transmission

Experimental FV3 infections have been induced by water bath exposure (Brenes et al. 2014b; Duffus et al. 2008; Harp & Petranka 2006; Hoverman, Gray & Miller 2010; Pearman et al. 2004; Waltzek et al. 2014), ingestion of infected tissue through necrophagy or cannibalism (Harp & Petranka 2006; Pearman et al. 2004), cohabitation with infected individuals (Brenes et al. 2014a; Harp & Petranka 2006), exposure to sediment (Harp & Petranka 2006) and injection of viral preparations (Johnson, Pessier & Jacobson 2007; Tweedell & Granoff 1968). Animals that are subclinically infected or that survive infection may become carriers of FV3 creating a reservoir for the disease. For example, FV3 was recovered in experimentally infected *S. albus* survivors 22 days post infection (dpi) (Waltzek et al. 2014). Transmission may also occur through contaminated fomites such as nets and shoes (Gray & Chinchar 2015). Vertical transmission of FV3 has not been demonstrated in any species (Miller, Gray & Storfer 2011).

Using a *X. laevis* experimental model, Robert et al. (2011) showed that FV3 released in water by infected adults can infect adult and larval stages within 3 hours of exposure (Robert et al. 2011). The average FV3 titre in ponds where a mortality event of *L. sylvaticus* tadpoles occurred was  $10^{2.46}$  PFU/mL and was higher than in ponds without observed mortality events ( $10^{-0.34}$  PFU/mL) (Hall et al. 2016). Virus was detectable in the pond water samples at least 2 weeks before and 5 weeks after the onset of mortality (Hall et al. 2016).



Ingestion of the virus typically results in faster mortality than exposure in water (Hoverman, Gray & Miller 2010; Pearman et al. 2004). For example, healthy *L. sylvatica* tadpoles exposed (but separated by a mesh screen) to moribund tadpoles collected during a local ranaviral die-off died within 4 dpi with onset of death accelerated when tadpoles were allowed to scavenge on carcasses of infected tadpoles (Harp & Petranksa 2006). Pearman et al. (2004) found that *R. latastei* tadpoles that cannibalized virus-infected carcasses experienced 30% greater mortality compared to tadpoles that were exposed to virions in a water bath (Pearman et al. 2004).

Interclass transmission of FV3 has been demonstrated. Brenes et al. (2014) showed cohabitating healthy and infected aquatic vertebrates from different classes (amphibian: *H. chrysoscelis*; reptile: *T. scripta elegans*; and fish: *G. affinis*) resulted in transmission of a FV3-like virus (Brenes et al. 2014a). A second study showed that bath challenges with FV3-like viruses isolated from a chelonian, fish and anuran resulted in infection of 2 of 5 fish species and 2 of 3 turtle species (Brenes et al. 2014b).

Water temperature is suggested to influence transmission of FV3 but depends on the host species (Gray & Chinchar 2015). FV3 bath challenges of *S. albus* resulted in a mean mortality rate of 42.5% of sturgeon maintained at 23°C whereas no mortality was observed among sturgeon maintained at 17°C although they were infected (Stilwell et al. 2022). This finding suggests that fish exposed at lower temperatures harbor the virus and could potentially serve as viral reservoirs until conditions become favourable for viral replication and transmission (Stilwell et al. 2022). Bayley et al. (2013) reported >96% mortality of *R. temporaria* tadpoles exposed to FV3 and FV3-like virus at 20°C but <32% mortality when exposed at 15°C (Bayley, Hill & Feist 2013). *T. scripta elegans* infected with FV3-like virus experienced 100% mortality at 22°C but 50% mortality at 28°C (Allender et al. 2013b).

It is thought that the worldwide movement of animals coupled with the broad host range of these viruses is responsible for the spread of FV3 and FV3-like viruses (Chinchar & Waltzek 2014; Owen 2022; Sriwanayos et al. 2020). Global transport of subclinically infected individuals may also be contributing to its spread (Gray & Chinchar 2015).

## Infectious dose

### Fish

In an experimental bath challenge, juvenile *S. albus* (mean weight 40 g) exposed to either  $1.3 \times 10^6$  TCID<sub>50</sub>/mL or  $1.3 \times 10^5$  TCID<sub>50</sub>/mL FV3 for 1 hour resulted in 90% mortality between 8–19 dpi (Waltzek et al. 2014). Bath exposure of *S. albus* (weight 14.4–50 g) for 1 hour to  $2.14 \times 10^6$  TCID<sub>50</sub>/mL FV3 resulted in  $52.5 \pm 13.2\%$  cumulative survival when fish were maintained at 23°C while no morbidity or mortalities were observed in fish maintained at 17°C (Stilwell et al. 2022). In the same study, bath exposure of *S. albus* (weight 18–57 g) for 1 hour to  $4.0 \times 10^4$  TCID<sub>50</sub>/mL FV3 at 23°C induced infection with clinical signs but no mortalities (Stilwell et al. 2022). The same experiment conducted at 17°C also induced infection in *S. albus* but no clinical signs or mortality were observed (Stilwell et al. 2022). Bath exposure of *E. lucius* fry (mean weight 0.03 g) to  $1 \times 10^4$  TCID<sub>50</sub>/mL FV3 for 2 hours resulted in infection (Bang Jensen, Kjær Ersbøll & Ariel 2009). Bath exposure of 5 fish species (fingerlings, mean 5–10 cm length) with  $10^3$  PFU/mL FV3-like viruses for 3 days was sufficient to induce infection and mortality (Brenes et al. 2014b).



*Amphibians*

Doses of  $10^2$ – $10^6$  PFUs of FV3 and FV3-like viruses were shown to be sufficient to induce infection in tadpoles (Duffus et al. 2008; Pearman et al. 2004; Pearman & Garner 2005; Robert et al. 2005; Tweedell & Granoff 1968). For example, Tweedell and Granoff (1968) showed that *L. pipiens* embryos and tadpoles were killed by injection with 900 PFU FV3 (Tweedell & Granoff 1968). Injection of tadpoles and young frogs (weight 20 g) of *L. grylio* with  $10^5$  TCID<sub>50</sub>/mL FV3 resulted in 90–100% mortality at 14 dpi (Zhang et al. 2001). Recent metamorphs of *L. sylvatica*, *L. pipiens* and *H. regilla* injected with  $3.1 \times 10^5$  FV3/animal induced 100% mortality by 4–12 dpi (Schock et al. 2008). Mortality was observed in tadpoles after 2 and 4 days following oral inoculation or 3-day bath exposure, respectively, with  $10^6$  PFUs FV3 (Hoverman, Gray & Miller 2010). Frog species bath exposed to  $10^3$  PFUs/mL FV3 for 3 days at hatchling, larval and metamorph stages suffered infection and mortality (Haislip et al. 2011; Hoverman et al. 2011). Bath exposure of *R. latastei* tadpoles to  $4.3 \times 10^6$  PFU/mL and  $4.3 \times 10^2$  PFU/mL FV3 for 24 hours caused 100% mortality by 4 and 12 dpi, respectively (Pearman et al. 2004).

*Reptiles*

*P. sinensis* (weight 4–6 g) intramuscularly injected with  $3 \times 10^5$  TCID<sub>50</sub>/mL FV3 or bath exposed to  $3 \times 10^5$  TCID<sub>50</sub>/mL FV3 for 2 hours exhibited 43% and 29% mortality, respectively (Chen, Zheng & Jiang 1999). *T. ornata ornata* and *T. scripta elegans* intramuscularly injected with  $10^5$  TCID<sub>50</sub>/mL FV3 became infected and showed clinical signs 8 dpi (Johnson, Pessier & Jacobson 2007).

**11.2.3 Pathogenesis****Tissue tropism***Fish*

In sturgeon, FV3 targets the skin, fin, gill, barbel, olfactory epithelium, heart, meningeal and pericardial lymphomyeloid tissue, liver, spleen, stomach, intestine and kidney (Stilwell et al. 2022).

*Amphibians*

In amphibians, FV3 infects the kidney, liver, pancreas, oesophagus, spleen, small intestine, heart, muscle, skin and gill (Chinchar, Waltzek & Subramaniam 2017; Miller et al. 2015; Miller et al. 2007; Robert et al. 2011).

*Reptiles*

In turtles, FV3 is present in the oral cavity, oesophagus, spleen, liver, kidney, stomach and intestine (Johnson, Pessier & Jacobson 2007; Johnson et al. 2008). FV3 was detected in skin, lungs, liver, spleen, kidney, small intestine, heart, nasal glands, mucosa and muscle of infected lizards (Behncke et al. 2013; Peiffer et al. 2019; Stohr et al. 2013). In snakes, FV3 was found in the kidneys, liver, spleen, nasal epithelium, skin and oral mucosa (Hyatt et al. 2002).

**Tissue titre***Fish*

In a bath challenge trial with  $1.3 \times 10^5$  or  $1.3 \times 10^6$  TCID<sub>50</sub>/mL FV3, the virus concentration in pooled spleen and kidney tissue from *S. albus* that died (mean weight 40 g) ranged from  $3.1 \times 10^7$ – $6.7 \times 10^8$  TCID<sub>50</sub>/g (Waltzek et al. 2014). Moribund or dead *S. albus* (weight 14.4–50 g) bath infected with  $2.14 \times 10^6$  TCID<sub>50</sub>/mL FV3 and held at 23°C had mean viral loads in pooled internal tissue homogenates (liver, kidney, spleen, heart and pericardial lymphomyeloid tissue) of  $1.2$ – $6.3 \times 10^5$  copies/μL by qPCR and  $63$ – $1 \times 10^6$  TCID<sub>50</sub>/mL by virus isolation (Stilwell et al. 2022).

Surviving *S. albus* 28 dpi recorded 5–6.3 FV3 copies/ $\mu\text{L}$  by qPCR (Stilwell et al. 2022). *S. albus* (weight 18–57 g) bath infected with  $4.0 \times 10^4$  TCID<sub>50</sub>/mL FV3 and held at 17°C or 23°C yielded maximum viral loads in pooled internal tissue homogenate at 7 dpi of 31.5 and  $7.9 \times 10^4$  copies/ $\mu\text{L}$ , respectively (Stilwell et al. 2022).

The average FV3 load in pooled liver and kidney tissue (0.25  $\mu\text{g}$ ) of experimentally infected fingerling fish *G. affinis* and *I. punctatus* was 11.3 PFU and 5.2 PFU, respectively (Brenes et al. 2014b).

#### Amphibians

The FV3 load in liver tissue from naturally-infected *L. sylvaticus* tadpoles was  $1.29\text{--}5.04 \times 10^8$  PFU (O'Connor, Rittenhouse & Brunner 2016). FV3 concentrations in the liver-kidney homogenate of dead wild *L. sylvaticus* tadpoles were 40–460 PFU/ $\mu\text{g}$  gDNA (Wheelwright et al. 2014). The FV3 load in liver and kidney homogenates from bath infected amphibian species were  $3.8 \times 10^4$  PFU/ $\mu\text{g}$  gDNA in *A. maculatum*,  $4.8 \times 10^3$  PFU/ $\mu\text{g}$  gDNA in *H. chrysoscelis*,  $2.3 \times 10^4$  PFU/ $\mu\text{g}$  gDNA in *L. clamitans* and  $7.2 \times 10^5$  PFU/ $\mu\text{g}$  gDNA in *L. sylvaticus* (Brand et al. 2016).

#### Reptiles

The FV3 load in experimentally infected turtles *A. ferox* and *G. pseudogeographica* was 0.8–760 PFU and 2.6 PFU, respectively (Brenes et al. 2014b). The median FV3 copy number from blood samples of infected *T. carolina carolina* was  $2 \times 10^3$  copies (range:  $0\text{--}2.4 \times 10^4$ ) and from oral swab samples was  $2.5 \times 10^3$  copies (range:  $0\text{--}3 \times 10^4$ ) (Allender et al. 2013a). *T. scripta elegans* experimentally infected with FV3 reported  $6.19 \times 10^5\text{--}3.37 \times 10^{10}$  copies/g of tongue,  $1.76 \times 10^{10}\text{--}2.39 \times 10^{11}$  copies/g of skeletal muscle,  $3.27 \times 10^9\text{--}2.32 \times 10^{10}$  copies/g of lungs,  $1.89 \times 10^{10}\text{--}3.72 \times 10^{10}$  copies/g of heart,  $1.32 \times 10^9\text{--}2.85 \times 10^9$  copies/g of liver,  $6.43 \times 10^9\text{--}1.77 \times 10^{11}$  copies/g of spleen,  $4.52 \times 10^8\text{--}1.31 \times 10^{10}$  copies/g of ovary and  $1.77 \times 10^9\text{--}5.69 \times 10^{10}$  copies/g of kidney (Allender et al. 2013b).

### 11.2.4 Diagnosis

#### Clinical signs

Clinical disease is often acute and can affect a high proportion of the population, resulting in mass mortalities (Gray & Chinchar 2015; Miller et al. 2015). Affected individuals typically present with haemorrhages, oedema and necrosis but clinical signs can vary with host species (Miller et al. 2015).

Subclinical FV3 infection has been reported in fish, amphibians and reptiles (Allender et al. 2013b; Brenes et al. 2014a; Brenes et al. 2014b; Carstairs, Kyle & Vilaça 2020; Duffus et al. 2008; Gray, Miller & Hoverman 2009; Harp & Petranksa 2006; Miller, Gray & Storfer 2011; Pearman et al. 2004; Soto-Azat et al. 2016). Multiple extrinsic (e.g. temperature, agricultural chemicals, host density and environmental stress) and intrinsic (e.g. spawning, age, developmental stage, health and immune competence) factors likely determine whether a given infection will be subclinical or result in clinical disease (Chinchar, Waltzek & Subramaniam 2017; Gray & Chinchar 2015).

Secondary infections are often found in FV3-infected individuals (Behncke et al. 2013; Miller, Gray & Storfer 2011; Miller et al. 2007; Stohr et al. 2013).

#### Fish

Infected sturgeon can display haemorrhagic lesions externally on the skin and fins, lethargy, erratic swimming and loss of buoyancy (Stilwell et al. 2022; Waltzek et al. 2014). An infected stickleback exhibited lethargy, disorientation, bright red and swollen gills, fused filaments and remained near the surface although it is unclear whether these clinical signs are solely from FV3 infection or also from

the coinfection with myxozoan parasites (Mao et al. 1999). *O. marmorata* exhibited minor ulcers on the body and around the mouth (Prasankok, Chutmongkonkul & Kanchankhan 2005).

#### *Amphibians*

Mortality is often the only clinical finding reported in cases of FV3 disease in amphibians. Gross clinical signs may include erratic swimming, loss of buoyancy, lethargy, anorexia, swelling of the legs and body, erythema (redness) of the legs and ventrum, ecchymosis (red blotches) near the vent and/or urostyle, petechiation (pinpoint haemorrhages) or ecchymosis of the skin and irregular patches of discoloration on the skin. Cutaneous erosions and ulcerations are frequently seen in adults (Cunningham et al. 1996; Miller, Gray & Storfer 2011; Miller et al. 2015).

#### *Reptiles*

Respiratory distress, anorexia, oral necrotic plaques, oral ulcers, skin ulcers, haemorrhages and swelling of head, neck and legs have been reported in infected turtles and tortoises (Miller et al. 2015). Other clinical signs observed include lethargy, red necks, nasal and ocular discharge, hyphema, conjunctivitis and palpebral oedema (Chen, Zheng & Jiang 1999; Johnson, Pessier & Jacobson 2007; Johnson et al. 2008; Kimble et al. 2017; McKenzie et al. 2019).

In infected lizards, skin lesions are common along with gray discoloration, ulceration and necrotising dermatitis (Stohr et al. 2013). Lethargy, anorexia and ocular discharge are also seen in some species (Behncke et al. 2013; Peiffer et al. 2019; Stohr et al. 2013).

#### **Pathology**

FV3 infection is characterised by systemic haemorrhaging and cellular necrosis, often resulting in organ failure within only a few days to 2–3 weeks of exposure (Miller, Gray & Storfer 2011). Necrosis of the hematopoietic tissues, vascular endothelium and epithelial cells and the presence of intracytoplasmic inclusion bodies are common in all hosts (Allender et al. 2013b; Bayley, Hill & Feist 2013; Cunningham et al. 1996; Johnson et al. 2008; Miller et al. 2015; Waltzek et al. 2014).

#### *Fish*

Microscopic lesions among infected sturgeon included necrosis of lymphohematopoietic and endothelial cells with eosinophilic intracytoplasmic inclusion bodies in the spleen, kidney, liver, and pancreas (Waltzek et al. 2014). Marked destruction of the spleen, haemorrhages in the liver, swim bladder and gill, empty gut and enlarged kidneys, spleen, liver and intestine have been observed (Stilwell et al. 2022; Waltzek et al. 2014).

#### *Amphibians*

In amphibians, haemorrhage and necrosis are present, especially in the spleen, kidney and liver (Miller et al. 2015). Necrosis has also been reported in the brain, lungs, heart, pancreas, intestines, gills, nasal tissue, thymus, trachea, muscle, epidermis and periosteum. Necrosis may present as generalized friable organs or as discrete pale foci scattered throughout an organ (Miller et al. 2015). Inclusion bodies that range from eosinophilic to basophilic are present in areas of necrosis (Miller et al. 2007). Tadpoles may also show pale livers and kidneys (Hoverman, Gray & Miller 2010). In metamorphs and adults, other pathological signs may include enlarged gall bladder, spleen or liver, mottled heart and kidneys and empty gut (Miller, Gray & Storfer 2011; Miller et al. 2007; Une et al. 2009b; Zhang et al. 2001).

### *Reptiles*

In turtles and tortoises, haemorrhage and necrosis were detected in the kidney, liver, spleen, pancreas, oesophagus, oral and nasal cavity, bone marrow, trachea, lungs and gastric tract (Chen, Zheng & Jiang 1999; Johnson et al. 2008; McKenzie et al. 2019; Peiffer et al. 2019). Multisystemic fibrinoid vasculitis, myositis, enlarged spleen, pale liver, empty gut, oedema of the gastrointestinal tract and petechiae of the tongue and kidneys are also observed (Allender et al. 2013b; Johnson, Pessier & Jacobson 2007; Johnson et al. 2008; McKenzie et al. 2019; Peiffer et al. 2019). Basophilic intracytoplasmic inclusions are sometimes present in the liver, bone marrow, adrenal tissue, oral and nasal mucosa and epidermis (Peiffer et al. 2019).

Systemic haemorrhages and necrosis have been described in infected lizards, especially in the liver, spleen, gastrointestinal and respiratory tracts (Behncke et al. 2013; Wirth et al. 2018). Ulcerative dermatitis and enlarged and pale livers have also been detected (Behncke et al. 2013; Stohr et al. 2013).

### **Testing**

Chapter 2.1.3 of the WOA *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 (Hyatt et al. 2000; Mao, Hedrick & Chinchar 1997; Mao et al. 1996) and real-time PCR (Allender et al. 2013a; Grant et al. 2019; Leung et al. 2017; Picco, Brunner & Collins 2007; Stilwell et al. 2018) can be used to detect FV3 and FV3-like viruses. *In situ* hybridisation, immunohistochemistry, electron microscopy and histology methods are also available (Miller et al. 2015).

### **11.2.5 Treatment**

There is no treatment currently available for FV3 infections (Gray & Miller 2013).

### **11.2.6 Control and prevention**

General control measures to prevent infections in aquaculture facilities include screening of individuals as FV3-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 et al. 2015; Miller, Gray & Storfer 2011; OIE 2021d). The disinfectants Chlorhexidine/Nolvasan® (0.75% and 2.0%), sodium hypochlorite/bleach (3% and 5%) and potassium peroxymonosulfate product/Virkon S® (1%) are effective at inactivating ranavirus after 1 minute exposure time (Bryan et al. 2009). FV3 is also sensitive to treatment with chloroform and 5-iodo-2-deoxyuridine (Chen, Zheng & Jiang 1999). There have been investigations into possible preventative treatments. For example, pre-treatment of cells with antisense morpholino oligonucleotides targeted to the major capsid protein of FV3, that were infected 24 hours later with FV3, showed a 90% reduction in virus titre (Sample et al. 2007).

### 11.2.7 Impact of the disease

FV3 and FV3-like viruses cause high levels of morbidity and mortality among commercially and ecologically important amphibian, fish and reptile species (Chinchar, Waltzek & Subramaniam 2017).

For example, the 4 FV3 outbreaks in *S. albus* at a hatchery in Missouri, USA, resulted in economic losses of >US\$400,000 (Stilwell et al. 2022). The acute disease duration of FV3 can lead to disease events occurring in wild populations without being detected and result in population declines (Chinchar & Waltzek 2014; Earl & Gray 2014; Teacher, Cunningham & Garner 2010). This would have serious consequences on endangered or geographically constrained populations. From experimental trials and the epidemiology of ranaviruses overseas, it is estimated the most likely outcome of a new ranavirus in Australia would be unpredictable local epidemics (WHA 2020).

### 11.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon or their reproductive material as import is not permitted.

There are biosecurity measures to manage the risk of *megalocytiviruses*, another genus in the *Iridoviridae* family, in imported ornamental fish for display purposes (see [Appendix B](#)).

## 11.3 Risk assessment

Based on [chapter 4](#) and the technical information about FV3 presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of FV3 meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 11.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for FV3 were that:

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that FV3 is present in all source countries.
- FV3 infects sturgeon that would be of a life stage exported to Australia.
- The prevalence of FV3 in farmed or wild sturgeon is unknown. Heavy mortalities (up to 90–100%) have been reported in 3 sturgeon hatcheries in the USA ((Stilwell 2017) cited in (Stilwell et al. 2022))(Waltzek et al. 2014).
- The prevalence of FV3 in other farmed or wild fish species is unknown.
- There are no reports of FV3 associated with sturgeon reproductive material or other fish eggs. In the absence of evidence it will be assumed that sturgeon reproductive material can be infected.
- FV3 can survive in water for extended periods.
- The viral load of FV3 in infected imported live sturgeon or their reproductive material is likely to be sufficient to cause infection in susceptible species.
- Inspection may detect sturgeon showing clinical signs of infection with FV3 and remove them before export. Sturgeon with mild or no clinical signs would be unlikely to be detected.

## Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of FV3 was estimated to be:

- Imported live sturgeon—**Moderate**.
- Imported sturgeon reproductive material—**Moderate**.

### 11.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for FV3 were that:

- FV3 can be transmitted horizontally via water, cohabitation, ingestion of infected tissues, contaminated fomites and can remain infectious in water and sediment for an extended period.
- FV3 would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- FV3 can infect a wide range of fish, amphibians and reptiles with some species present in Australia including fish *G. affinis*, *Oxyeleotris* species, reptiles *M. viridis*, *P. vitticeps*, and *T. scripta elegans*. *Gambusia* species, some *Oxyeleotris* species and *T. scripta elegans* are considered noxious species or pests in some states and territories.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are not susceptible to FV3.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the range 16–26°C reported for the onset of FV3 disease in sturgeon (Waltzek et al. 2014).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infected sturgeon will be certain to be exposed to viable FV3.
- Introduction of FV3 into the wild may occur by direct release of imported live sturgeon or its associated wastes from the aquaculture facility into natural waters. This would be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for fish escapes or waste management that would exclude FV3 from discharges.
- Wild susceptible species would be less abundant than susceptible species in aquaculture facilities. Despite this, wild susceptible species would be expected to be exposed to FV3 released into natural waters due to its wide host range.

## Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure to each exposure group to FV3 in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

The partial likelihood of exposure to each exposure group to FV3 in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**Moderate**.

- Wild susceptible species—**Moderate**.

### 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 FV3 in **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Low**.

The partial annual likelihood of entry and exposure of each exposure group to FV3 in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**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 FV3 were that:

- FV3 can be transmitted horizontally via water, cohabitation, ingestion of infected tissues, contaminated fomites and can remain infectious in water and sediment for an extended period. Interclass transmission between amphibians, fish and reptiles can also occur.
- It is expected that susceptible species in contact with FV3-infected animals would receive an infectious dose.
- Fish that survive FV3 infections may become carriers and sources of the virus.
- FV3 can infect a wide range of fish, amphibians and reptiles with some species *present in* Australia including fish *G. affinis*, *Oxyeleotris* species, reptiles *M. viridis*, *P. vitticeps*, and *T. scripta elegans*. *Gambusia* species, some *Oxyeleotris* species and *T. scripta elegans* are considered noxious species or pests in some states and territories.
- FV3 establishment, following a given quantity of FV3 entering the environment of an exposure group, is likely for farmed susceptible species due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- It is unlikely that polycultured species will be infected as aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are not susceptible to FV3.
- If infected live sturgeon or reproductive material in an aquaculture facility were moved to another aquaculture facility in Australia it is likely that FV3 would establish in these facilities.
- Each state and territory have translocation protocols for aquaculture animals but may not include consideration of FV3.
- If FV3 were to establish on a farm it could spread to neighbouring farms and wild populations through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of FV3 be suspected and response measures initiated



immediately. However, FV3 is effectively transmitted through water and can persist in the environment and farms which share a common water source or equipment with an infected population may be exposed to FV3.

- The likelihood of FV3 spread from farms to wild populations or neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however FV3 could spread this way. FV3 could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.
- If one or more index cases of FV3 were to occur in the wild, establishment and spread would be more likely than on a farm because the wide range of susceptible animals increases the opportunities for transmission. Further, because FV3 can survive in the environment, it could persist until susceptible hosts were to encounter it.
- The likelihood of FV3 in a wild population spreading to its natural geographic limits is greater than for other hazards with limited host ranges, for example, typical *A. salmonicida*, and would be more likely than for those hazards which cannot survive outside of a host for long periods. The ability of fish, amphibians and reptiles to be subclinically infected with FV3 also aids its spread.
- If FV3 were to establish in the wild, especially in waters around aquaculture facilities, it may easily spread to farms through water intake due to FV3 being able to survive in the environment and being transmissible through water. In the absence of effective biosecurity measures, wild infected fish, amphibians or reptiles may be transferred into the farms through the inlet water channels. There are also susceptible amphibian and reptile species which can enter farms through movement across short distances of land and could potentially carry FV3 with them.

### Conclusion

Based on these considerations and using the descriptors in Table 4, the partial likelihood of establishment and spread of FV3 in each exposure group for the outbreak scenario was estimated to be:

- Farmed susceptible species—**Moderate.**
- Wild susceptible species—**High.**

### Determining adverse impacts resulting from the outbreak scenario

The factors considered relevant when determining the adverse impacts resulting from establishment and spread of FV3 were that:

#### *Direct effects*

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

- Ranaviruses are highly infectious and can cause rapid, fatal disease in a wide range of ectothermic vertebrates.
- Sturgeon are susceptible to FV3 and there is high mortality associated with infection. The establishment of a sturgeon industry in Australia would be significantly affected by an outbreak of FV3.
- Production losses in sturgeon aquaculture would be significant considering sturgeon require >3 years to reach sexual maturity.



- There are no other farmed fish species in Australia that are known to be susceptible to FV3.
- FV3 would not be expected to impact wild fisheries in Australia. There are limited reports of FV3 in wild fish and no reports of associated declines in catch rates or mortalities.
- Based on the previous reports of the impact of FV3 in sturgeon, FV3 establishment and spread in Australia would be expected to cause minor impacts at the state or territory 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*

- FV3 has a wide host range, including amphibians, fish and reptiles and outbreaks can lead to significant mortalities and die-off events.
- Natural ranavirus infections are known from most of the major families of Anura (frog) and Caudata (salamander) (OIE 2021d).
- The direct impact of FV3 establishment and spread on the living environment is expected to be significant at the national level.

*Indirect effects*

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

- Infection of amphibians with ranaviruses, including FV3, is listed as a notifiable disease by WOA and is included on Australia's *National list of reportable diseases of aquatic animals*. Although infection of fish with FV3 is not listed in Australia, state and territory governments would be expected to report on the presence of FV3.
- If FV3 was confirmed at a farm, 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 FV3 was confirmed in the environment, eradication would be near impossible as the agent is able to persist in water and sediments.
- If a movement restriction area were put in place for an outbreak of FV3, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- Eradication and control of FV3 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*

- Industries supplying inputs into the affected sturgeon industry may suffer losses. For example, where farm production is halted or decreased, feed companies would be impacted by reduced feed purchases.
- FV3-infected fish may show gross signs which could affect their marketability.
- FV3 establishment and spread would likely have a minor impact at the local 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 of amphibians with ranaviruses, including FV3, is a WOAHL-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to FV3 to avoid the possible introduction of FV3.
- If FV3 were to become established, Australia could use zoning to maintain access to international markets for live susceptible species, including sturgeon and, if required, non-viable product.
- The impacts of FV3 establishment and spread on international trade are likely to be minor at the local level.

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

- FV3 has a wide host range including amphibians, fish and reptiles. Natural ranavirus infections are known from most of the major families of Anura (frog) and Caudata (salamander) (OIE 2021d).
- A conservative approach has been adopted when considering the susceptibility of native species, particularly those that are endangered or threatened. If FV3 were to cause clinical disease in an endangered species, it could result in a significant impact on the survival of that species.
- It is therefore assumed that FV3 and FV3-like viruses may impact on native populations of amphibians and reptiles in Australia. Currently, more than 17 species of frogs of the families *Hyalidae* and *Myobatrachidae* and 9 reptiles are listed as critically endangered ([EPBC Act List of Threatened Fauna](#)). Ten frog species and 20 reptiles are listed as endangered and 13 frog species and 31 reptiles as vulnerable.
- The impact of FV3 establishment and spread on the biodiversity of the environment is considered 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*

- Areas where amphibians susceptible to FV3 are present could be affected by mortalities and movement restriction areas put in place which may impact on social amenity.
- In local areas where aquaculture of susceptible species is a major industry, a FV3 outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of FV3 establishment and spread are expected to be minor at the state or territory level.

Table 17 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of FV3. 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 17 Overall impact of establishment and spread of FV3 for the outbreak scenario**

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	State or territory	Minor	D
	The environment (native animals/plants, and non-living environment)	National	Significant	F
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)	Local	Minor	B
	Economic (international trade effects)	Local	Minor	B
	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 FV3 was estimated to be **high**.

**Determination of likely consequences of the outbreak scenario**

The likely consequences of the outbreak scenario for FV3 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:

- Farmed susceptible species—**High**.
- Wild susceptible species—**High**.

**11.3.5 Determination of the partial annual risk**

The partial annual risk of FV3 entry, establishment and spread from **imported live sturgeon** 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

The partial annual risk of FV3 entry, establishment and spread from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

**11.3.6 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 FV3 was found to be:

- Imported live sturgeon—**High**.

- Imported sturgeon reproductive material—**High**.

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

## 11.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for FV3 in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 11.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 18 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of FV3 but which on their own do not achieve Australia's ALOP for FV3 in imported live sturgeon or their reproductive material.

**Table 18 Biosecurity measures that on their own do not achieve Australia's ALOP for FV3**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Disease-free stocks	<b>Yes:</b> Determination of FV3 freedom would need to be to a standard consistent with that recommended by the World Organisation for Animal Health (WOAH), or equivalent.
2	Post-arrival quarantine (PAQ)	<b>Yes:</b> The stress of transport can induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
3	Post-arrival batch testing	<b>Yes:</b> There is PCR and real-time PCR testing available to detect FV3 (Leung et al. 2017; Mao, Hedrick & Chinchar 1997; Mao et al. 1996; Picco, Brunner & Collins 2007). Under this scenario, testing is conducted under departmental control and oversight.

### 11.4.2 Biosecurity measures that in combination achieve Australia's ALOP

A combination of biosecurity measures 1, 2 and 3 when applied to **imported live sturgeon** would reduce the likelihood of entry of FV3 from **moderate** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1, 2 and 3 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of FV3 from **moderate** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

# 12 Infectious haematopoietic necrosis virus

## 12.1 Background

Infectious haematopoietic necrosis virus (IHNV), also known as *Novirhabdovirus salmonid*, is the aetiological agent of infectious haematopoietic necrosis (IHN) (WOAH 2022b). IHNV is classified in the genus *Novirhabdovirus* and family *Rhabdoviridae* (WOAH 2022b). IHN is primarily a disease of salmonids, but other species of freshwater and marine fish are also susceptible to the virus (Groff & LaPatra 2000).

Serious losses in salmonids due to IHNV were first reported from North America in 1953 (Rucker et al. 1953). Infections with IHNV have since been detected in Africa, the Americas, Asia and Europe (WOAH 2022b).

Infection with IHNV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (WOAH 2022b) and is listed on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). Australia has a long history of passive surveillance and a strong system in place to detect incursions. IHNV is considered exotic to Australia.

## 12.2 Technical information

### 12.2.1 Agent properties

IHNV is a bullet-shaped virion, 150–190 nm × 65–75 nm in dimension, that encapsulates a single-stranded, negative-sense RNA segment ((Wolf 1988) cited in (WOAH 2022b)). IHNV is classified by the International Committee on Taxonomy of Viruses as a member of the genus *Novirhabdovirus* in the family *Rhabdoviridae* (ICTV 2022).

Sequence analysis of the glycoprotein found on the surface of mature virions has classified IHNV isolates into 5 major genogroups that are more closely related to geographical location rather than host species (Enzmann et al. 2005; Hsu, Engelking & Leong 1986; Kurath et al. 2003; Nishizawa et al. 2006a). Antigenic studies using polyclonal anti-glycoprotein sera have shown that all isolates of IHNV form a single serotype (Engelking, Harry & Leong 1991).

IHNV can survive outside the host and remains infectious for longer in freshwater compared with seawater (Kell, Wargo & Kurath 2014; Toranzo & Hetrick 1982). For example, IHNV was detected in effluent water from an adult *Oncorhynchus mykiss* (rainbow trout) holding pond at a concentration of  $2 \times 10^1$  PFU/mL and  $3 \times 10^{-4}$  PFU/mL was detected in water from a river in Oregon, USA where IHNV was enzootic (Watanabe, Fryer & Rohovec 1988). IHNV isolates were shown to survive in 25°C filtered river water for 35–49 days and >65 days when the water was at 4°C and 10°C (Joiner et al. 2021). In unfiltered water, survival was lower at all temperatures tested although could still be >65 days at 4°C (Joiner et al. 2021). IHNV also survives freezing at –20°C and –80°C (Burke & Mulcahy 1983; Garver et al. 2013a; Hostnik et al. 2002; LaPatra et al. 1990; McClure et al. 2008).

Water temperature is an important factor in the virulence of IHNV. Clinical signs of IHNV infection usually occur in fish kept at water temperatures of 8–15°C and mortality can occur from 3–18°C

(Dixon et al. 2016; LaPatra 1998; WOA 2022b). Experimental studies showed that IHNV inactivation rates in water are reduced at lower water temperatures (Kell, Wargo & Kurath 2014; Toranzo & Hetrick 1982; Yoshimizu et al. 2005). Outbreaks rarely occur once water temperatures exceed 15°C although chronic outbreaks above 17°C have been observed in juvenile *Oncorhynchus tshawytscha* (chinook salmon) (Foott et al. 2006; WOA 2022b).

IHNV can be inactivated by UV exposure (Garver et al. 2013a), the microbial community in the water (Garver et al. 2013a; Kamei et al. 1987), and common disinfectants such as sodium hypochlorite, iodophor, benzalkonium chloride, saponated cresol, formaldehyde and potassium permanganate solution (Yoshimizu et al. 2005).

## 12.2.2 Epidemiology

### Host range

Species which fulfil the criteria for listing as a species susceptible to infection (N=natural exposure; E=experimental exposure) with IHNV in accordance with chapter 1.5 of the WOA Code (WOA 2022b) include:

- *Esox lucius*<sup>N, E</sup> (northern pike) (Cabon et al. 2020; Dorson et al. 1987)
- *Oncorhynchus clarkii*<sup>N</sup> (cutthroat trout) (LaPatra 1998)
- *Oncorhynchus keta*<sup>N</sup> (chum salmon) (LaPatra 1998)
- *Oncorhynchus kisutch*<sup>N</sup> (coho salmon) (LaPatra 1998)
- *Oncorhynchus masou*<sup>N</sup> (masu salmon) (LaPatra 1998)
- *Oncorhynchus mykiss*<sup>N</sup> (rainbow trout) (Amend, Yasutake & Mead 1969; LaPatra 1998)
- *Oncorhynchus nerka*<sup>N</sup> (sockeye salmon) (Amend, Yasutake & Mead 1969; LaPatra 1998)
- *Oncorhynchus tshawytscha*<sup>N</sup> (chinook salmon) (LaPatra 1998)
- *Salmo marmoratus*<sup>E</sup> (marble trout) (Pascoli et al. 2015)
- *Salmo salar*<sup>N</sup> (Atlantic salmon) (LaPatra 1998)
- *Salmo trutta*<sup>N</sup> (brown trout) (LaPatra 1998)
- *Salvelinus alpinus*<sup>E</sup> (Arctic char) (LaPatra 1998)
- *Salvelinus fontinalis*<sup>N</sup> (brook trout) (LaPatra 1998)
- *Salvelinus namaycush*<sup>E</sup> (lake trout) (LaPatra 1998).

Other host species shown to be susceptible to infection with IHNV include (N=natural exposure; E=experimental exposure):

- *Acipenser transmontanus*<sup>E</sup> (white sturgeon) (LaPatra et al. 1995)
- *Anguilla anguilla*<sup>N, E</sup> (European eel) (Bergmann et al. 2003)
- *Aulorhynchus flavidus*<sup>N</sup> (tube-snout) (Kent et al. 1998)
- *Clupea pallasii*<sup>N</sup> (Pacific herring) (Kent et al. 1998)
- *Cymatogaster aggregate*<sup>N</sup> (shiner perch) (Kent et al. 1998)

- *Dicentrarchus labrax*<sup>E</sup> (European seabass) (Castric & Jeffroy 1991)
- *Lota lota*<sup>E</sup> (burbot) (Polinski et al. 2010)
- *Oncorhynchus gorbuscha*<sup>N</sup> (pink salmon) (LaPatra 1998)
- *Oncorhynchus rhodurus*<sup>N</sup> (Amago salmon) (LaPatra 1998)
- *Prosopium williamsoni*<sup>E</sup> (mountain whitefish) (LaPatra et al. 2016)
- *Salmo labrax*<sup>N</sup> (black sea salmon) (Nishizawa et al. 2006b)
- *Salvelinus leucomaenis*<sup>N</sup> (Japanese charr) (LaPatra et al. 2016)
- *Scophthalmus maximus*<sup>E</sup> (turbot) (Castric & Jeffroy 1991)
- *Sparus aurata*<sup>E</sup> (gilthead seabream) (Castric & Jeffroy 1991)
- *Thymallus thymallus*<sup>N</sup> (grayling) (Kolodziejek et al. 2008).

IHNV-positive RT-PCR results have been reported in a few species (N= natural exposure; E= experimental exposure), however no active infection has been demonstrated, including:

- *Cyprinus carpio*<sup>E</sup> (common carp) (Palmer & Emmenegger 2014)
- *Perca flavescens*<sup>E</sup> (American yellow perch) (Palmer & Emmenegger 2014).

Host susceptibility to IHNV is influenced by multiple factors including virus isolate, fish stock, species, age, size and life stage (Bergmann et al. 2003; Garver et al. 2013a). Salmonid fry and fingerlings are more susceptible to the virus than adult salmonids, except for spawners who are highly susceptible to IHNV (Dixon et al. 2016; LaPatra et al. 2016; WOA 2022b).

There is only one report of IHNV infecting live sturgeon. In experimental studies, larval *A. transmontanus* (60 days post-hatch, mean weight 0.5 g) were susceptible to IHNV via immersion challenge whereas juveniles (mean weight 50 g) were refractory to infection (LaPatra et al. 1995). Additionally, anti-IHNV sera was detected in adult *A. transmontanus* (4–6 years old) co-cultured with IHNV-infected *O. mykiss* in a raceway (LaPatra et al. 1995). There are no reports showing natural exposure of sturgeon to IHNV. WOA does not list any sturgeon as a susceptible host species but does list *A. transmontanus* as a species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to IHNV infection (WOA 2022c). Sturgeon have been reported to be possible vector species of IHNV under European Union legislation (Communities 2008).

### Geographical distribution

IHNV has been reported in Austria, Belgium, Canada, Chile, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Iran, Italy, Japan, the Republic of Korea, the Netherlands, North Macedonia, Poland, Republic of Türkiye, the Russian Federation, Slovenia, Spain, Switzerland, Taiwan and United States of America (USA) (Dixon et al. 2016; EURL for Fish and Crustacean Diseases 2021; OIE 2021b).

### Prevalence

#### Sturgeon

No reports of the prevalence of IHNV in farmed or wild sturgeon were found.

### Salmonids

IHNV is endemic among populations of wild salmonids along the west coast of North America (WOAH 2022b). For example, the prevalence of IHNV in spring-run, autumn-run and summer-run *O. tshawytscha* in the Columbia River Basin, USA from 2000–2012 was on average 27%, 25% and 13% (Hernandez et al. 2021). IHNV prevalence during 2000–2012 in *O. mykiss* and *O. kisutch* in the watersheds of Oregon, Washington and the Columbia River Basin, USA, were 24% and 4%, respectively (Breyta et al. 2017). Surveys of 2,564 wild juvenile *O. nerka* collected from the Strait of Georgia and Discovery Islands, Canada from 2010–2015 detected IHNV at 0% (2012, 2013 and 2015) to a maximum of 10.5% (2014) prevalence (Garver & Wade 2017).

In infected *O. mykiss* farms in Europe, the IHNV prevalence can be as high as 59% (Dixon et al. 2016). *S. trutta* collected in 2006–2008 from rivers in Kosovo had a IHNV prevalence of 25% (n=32 pools with 5 fish per pool) (Rexhepi et al. 2011). In a screening of 150 pooled *O. mykiss* samples from 50 farms in North Macedonia for IHNV, where each pool consisted of 3 fish, 1.3% (n=150) tested IHNV-positive (Cvetkovikj et al. 2020). In a 2006 survey of *O. mykiss* from the Namdae River, the Republic of Korea, 27.5% (n=80) of broodstock were found to be IHNV-positive (Jeon et al. 2011). In 2007 from the same location, 84.4% (n=32) of pooled *O. mykiss* fry were IHNV-positive as well as 1.3% (n=80) of broodstock. In 2008, 59.4% (n=32) of pooled *O. mykiss* fry were IHNV-positive (Jeon et al. 2011). IHNV prevalence in *O. mykiss* collected from 60 farms in Iran in 2015 was 14% (n=660) (Jalali et al. 2019).

### Other fish

IHNV was detected in *E. lucius* collected in 2016 from a wild pond in France at a prevalence of 23% (n=30) (Cabon et al. 2020).

IHNV was detected in 6 fish farms in 2017–18 in Finland (EURL for Fish and Crustacean Diseases 2021). In 2020, 24 new outbreaks of IHNV were reported in Europe with 21 in Germany, 1 in Croatia, 1 in Slovenia and 1 in France (EURL for Fish and Crustacean Diseases 2021). Additionally, in 2020 there was 1 IHNV-infected fish farm (subject to minimum control measures) in Austria (n=4862), 2 farms in Belgium (n=100), 23 farms in Germany (n=10,813), 12 farms in Italy (n= 816), and 23 farms in Slovenia (n=303) (EURL for Fish and Crustacean Diseases 2021).

## Mortalities

### Sturgeon

No reports of mortalities in farmed or wild sturgeon due to IHNV were found.

### Salmonids

Mass mortalities of up to 90% can occur in hatcheries infected with IHNV, typically with 100% of the animals infected (Australian Government Department of Agriculture 2019). There have also been reports of mortality in older fish such as 14–16 month wild *O. nerka* (weight 27–65 g) in Alaska (Burke & Grischkowsky 1984). Mortality typically occurs at water temperatures of 3–18°C (WOAH 2022b).

An IHNV outbreak in Canada from 2001–2003 affected 36 *S. salar* farms with mortality ranging from 20–94% and averaging 58% (Saksida 2006). Mortality was highest in the smolt populations and decreased with increasing size and age of the salmon (Saksida 2006). In 2012, IHNV was detected in 3 *S. salar* farms in Canada and caused mass mortality (no numbers given) (Garver et al. 2013a). An IHNV outbreak occurred in farmed yearling *O. mykiss* in Pengzhou, China in 2013 with fish suffering



>40% mortalities (Yu et al. 2016). IHN infection of farmed *O. mykiss* in Iran during 2015–2019 caused 20–90% cumulative mortalities in fry (Ahmadivand, Palić & Weidmann 2021; Ahmadivand et al. 2017). In 2019, Japan reported IHN in 5 prefectures infecting *O. masou*, *O. mykiss* and *O. rhodurus* and causing 1–50% mortality (NACA, OIE-RRAP & FAO 2019).

### Transmission

The transmission of IHN is primarily horizontal through direct contact with virus contaminated water or via cohabitation with IHN-infected fish (Bootland & Leong 1999). The virus is shed into the water from infected fish via sexual fluids and external mucus (Dixon et al. 2016; WOA 2022b). For example, naturally infected juvenile *O. tshawytscha* showing clinical signs shed  $10^3$  PFU/mL of virus into water during a 1 minute period (Foott et al. 2006). Experimentally infected *S. salar* (mean weight 350 g) started to shed IHN into water before the onset of clinical signs with an average peak shed rate of  $3.2 \times 10^7$  PFU/fish/hour 1–2 days prior to mortality (Garver et al. 2013a). Bath exposed *O. mykiss* (weight 1–3 g) shed IHN within 24 hours of infection with a peak shed rate of  $9.37 \times 10^5$  PFU/fish/day which was 3 days prior to the onset of mortality (Wargo et al. 2017). Fish that died during the experiment also shed detectable IHN after death, in some cases for up to 5 days, but it is unknown if the virus was infective (Wargo et al. 2017). IHN entry is primarily through the gills, fins and skin but tissues of the digestive system may also be involved if fish feed on infected fry (Drolet, Rohovec & Leong 1994; Harmache et al. 2006; Yamamoto & Clermont 1990).

There is insufficient evidence to demonstrate true vertical transmission. However, transmission from broodstock to progeny occurs as IHN has been detected in sexual fluids from broodstock (Meyers et al. 2003; Mulcahy, Pascho & Jené 1983) and disinfection of eggs has been shown to reduce IHN titres (Bovo et al. 2005a; Goldes & Mead 1995; Traxler et al. 1997).

There is some evidence that fish that survive IHN become carriers of the virus for extended periods (LaPatra et al. 2016). For example, IHN was detected in experimentally infected *O. tshawytscha* up to 39 days post infection (dpi) in the absence of clinical signs (Foott et al. 2006). *O. nerka* and *O. mykiss* survivors of laboratory exposures have shown IHN persistence for months to over one-year post-exposure (Drolet et al. 1995; Muller et al. 2015). IHN was detected in larval *A. transmontanus* for at least 9 dpi but it is unknown how long the fish remained infected (LaPatra et al. 1995).

Several possible vectors have been identified for IHN. For example, *Lepeophtheirus salmonis* (salmon lice) are capable of temporarily acquiring and transmitting IHN to healthy *S. salar* (Jakob, Barker & Garver 2011). IHN has also been isolated from freshwater invertebrates such as leeches, copepods and mayflies although their capacity to transmit virus is yet unknown (Dixon et al. 2016; Garver & Wade 2017; Mulcahy, Klaybor & Batts 1990).

Fomites can also transmit IHN. For example, IHN was found to remain infectious for several weeks after being dried on stainless steel and in various soil types highlighting the risk of mechanical transmission and persistence in a dry environment (Joiner et al. 2021).

Aquatic experts estimated the movement of live fish was the highest risk factor for spreading IHN with exposure to infected water also an important pathway (Oidtmann et al. 2014). Transmission of IHN has also been shown to increase with host density (Ogut & Reno 2005).

### Infectious dose

The infectious dose of IHN virus appears to vary depending on the isolate and the host species (Dixon et al. 2016). For example, bath exposure of *O. tshawytscha* (mean weight 1.37 g) to  $5.7 \times 10^3$  PFU/mL IHN virus for 1 minute or 10 minutes caused 30% and 70% prevalence of infection, respectively, by 19 dpi with low mortalities (Foott et al. 2006). Infections without clinical signs were induced in *O. tshawytscha* when bath exposed to  $>10^2$  PFU/mL IHN virus for 1 minute and clinical signs were observed when challenge doses were  $>10^4$  PFU/mL (Foott et al. 2006). Bath exposure of *O. mykiss* (mean weight 1.0 g) with  $1 \times 10^4$  PFU/mL for 1 hour could induce IHN virus infection with an onset of mortality at 5 dpi that ranged from 32–90% (Purcell et al. 2010). *O. mykiss* (0.2–13.1 g) and *O. nerka* (0.2–7.2 g) bath exposed to IHN virus at  $10^{1.9}$  PFU/mL for 12 hours resulted in cumulative mortalities of up to 88% and 100%, at 14 and 20 dpi, respectively (LaPatra et al. 1990). *O. mykiss* (mean weight 3.8 g) and *S. marmoratus* (mean weight 4.3 g) bath exposed to  $10^{7.55}$  TCID<sub>50</sub>/mL IHN virus solution for 4 hours resulted in 28.4% and 3.2% mortality, respectively, by 30 dpi (Pascoli et al. 2015).

*S. salar* (1 month old) bath exposed to  $10^5$  TCID<sub>50</sub>/mL IHN virus for 1 hour with one IHN virus isolate resulted in 100% mortality within 13 dpi whereas a different isolate only caused 50% mortality by 19 dpi (Yamamoto & Clermont 1990). Infection and mortality was induced in *S. salar* (mean weight 122 g) by bath exposure with 10 PFU/mL IHN virus for 1 hour (Garver et al. 2013a). *S. salar* (mean weight 122 g) bath exposed to  $10^4$  and  $10^2$  PFU/mL IHN virus for 1 hour resulted in cumulative mortalities of 32% and 34% with an average day to death of 23 and 33 dpi, respectively (Garver et al. 2013a). Experimental challenge of *S. salar* (mean weight 420 g) after a 1 hour bath exposure to IHN virus at a dose of  $4.56 \times 10^3$  PFU/mL induced mortality at 10 dpi with cumulative mortality reaching 44% at 51 dpi (Garver et al. 2013a).

Bath exposure of larval *A. transmontanus* (mean weight 0.5 g) to  $10^6$  PFU/mL of IHN virus for 1 hour resulted in 20% mortality by 14 dpi although the authors noted that such high viral concentrations never occur in aquaculture or natural conditions (LaPatra et al. 1995).

## 12.2.3 Pathogenesis

### Tissue tropism

IHN virus targets the haematopoietic tissue and is most commonly isolated from the kidney and spleen (WOAH 2022b). The virus has also been isolated from gills, pharynx, oesophagus, intestine, stomach, liver, brain, heart, thymus, pancreas, muscle, skin, fin, mucus, ovarian fluid and milt (Dixon et al. 2016; Drolet, Rohovec & Leong 1994; Harmache et al. 2006; LaPatra, Rohovec & Fryer 1989; Mulcahy et al. 1982; Yamamoto & Clermont 1990).

### Tissue titre

The viral load in fish with clinical signs can be very high, regularly up to  $10^8$ – $10^9$  PFU/g tissue and occasionally  $10^{10}$  PFU/g but the average titres are in the order of  $10^4$ – $10^6$  PFU/g (Dixon et al. 2016). *A. transmontanus* (mean weight 0.5 g) that died from an experimental challenge had IHN virus concentrations of greater than  $10^5$  PFU/g (LaPatra et al. 1995). IHN virus was not detectable at 24 hours post infection but 72% of the fish had IHN virus concentrations of  $10^2$ – $10^6$  PFU/g at 2–9 dpi (LaPatra et al. 1995).

Naturally infected juvenile *O. tshawytscha* showing clinical signs had a mean IHN virus concentration of 106 PFU/mL in their mucus (Foott et al. 2006). In naturally infected juvenile *O. tshawytscha* (mean weight 1.86 g) mucus preparations from dead fish had IHN virus concentrations ranging from  $10^{2.5}$  –

$10^{5.8}$  PFU/mL (LaPatra, Rohovec & Fryer 1989). In *O. mykiss* (mean weight 2.4 g), IHN was detected in mucus and gills from 24 hours after bath exposure and increased to levels near  $10^4$  PFU/mL by 48 hours post exposure and remained at this level for at least 2 days (LaPatra, Rohovec & Fryer 1989). The IHN concentration in tissues from juvenile *O. tshawytscha* bath exposed to  $7.0 \times 10^4$  PFU/mL for 1 hour was  $10^3$  PFU/g in the gill, skin and pooled kidney and spleen tissue at 3 dpi (Foott et al. 2006). Naturally IHN-infected *O. tshawytscha* (weight 25-82 g) showing clinical signs had a virus concentration of  $10^2$ – $10^6$  PFU/g in brain tissue and  $10^{1.3}$ – $10^{2.4}$  PFU/mL in mucus (LaPatra, Rohovec & Fryer 1989). The mean tissue titres in dead *S. salar* (mean weight 122 g) bath exposed to  $10^2$ – $10^4$  PFU/mL for 1 hour ranged from  $1.6 \times 10^5$ – $1.1 \times 10^7$  PFU/g (Garver et al. 2013a).

IHN concentrations in the brain of naturally infected pre-spawning and spawning female *O. nerka* were  $5.5 \times 10^2$  and  $9.3 \times 10^2$  PFU/g, respectively, compared with  $1.6 \times 10^4$  and  $2.2 \times 10^4$  PFU/g in the spleen,  $6.5 \times 10^3$  and  $3.1 \times 10^4$  PFU/g in the kidney and  $5.4 \times 10^3$  and  $2.7 \times 10^5$  PFU/g in the gill (Mulcahy et al. 1982). IHN-infected adult *O. mykiss* from a hatchery reported IHN mean concentrations of  $10^{3.6}$  PFU/mL for ovarian fluid,  $10^{2.5}$  PFU/g for gill, and  $10^{2.6}$  PFU/mL in mucus (LaPatra, Rohovec & Fryer 1989). Concentrations of IHN in tissues of *O. tshawytscha* broodstock following bath exposure to  $3.5 \times 10^5$  TCID<sub>50</sub>/mL of IHN ranged from  $1.50 \times 10^4$ – $4.68 \times 10^5$  PFU/g in pooled kidney and spleen,  $2.35 \times 10^6$ – $3.65 \times 10^8$  PFU/mL in ovarian fluid,  $1.25 \times 10^4$ – $1.78 \times 10^7$  PFU/g in gills and  $1.25 \times 10^4$ – $2.13 \times 10^5$  PFU/mL of plasma at 13–14 dpi (Arkush et al. 2004). The IHN concentration in the kidneys from naturally infected *O. nerka* broodstock ranged from  $1.0 \times 10^2$  –  $3.0 \times 10^6$  PFU/g (Traxler et al. 1997).

## 12.2.4 Diagnosis

### Clinical signs

IHN is characterised by various clinical signs including lethargy, random frenzied swimming events, darkening of the skin, pale gills, skin haemorrhages, distended abdomen and exophthalmia (WOAH 2022b). Surviving fish may exhibit spinal deformities (Australian Government Department of Agriculture 2019; Muller et al. 2015).

Depending on the species of fish, rearing conditions, water temperature and the virus isolate, outbreaks of IHN may range from explosive events of morbidity and mortality to chronic infections in fish (Winton 1991). Infected fish do not always display clinical signs, particularly adults and spawners (Foott et al. 2006; Mulcahy, Jené & Pascho 1984; Muller et al. 2015; Pascoli et al. 2015; Rexhepi et al. 2011; Traxler et al. 1997). Infected wild fish populations rarely show clinical signs (Rexhepi et al. 2011; Traxler et al. 1997). The incubation period from natural exposure to the virus to onset of clinical signs has been reported as short as 7 days (Saksida 2006).

### Pathology

IHN can cause pale liver, kidney and spleen, yellow mucus in the intestine, ascites, anaemia, empty gut, and petechial haemorrhage in the visceral mesenteries, swim bladder, adipose tissue, and peritoneum. The disease progresses to a degenerative necrosis of the haematopoietic tissues of the kidney, spleen, liver, pancreas and digestive tract ((Wolf 1988) cited in (LaPatra et al. 2016))(Bootland & Leong 1999; Dixon et al. 2016).

### Testing

Chapter 2.3.5 of the WOAH *Manual of diagnostic tests for aquatic animals* (WOAH 2022b) provides details of the methods currently available for surveillance and confirmatory diagnosis of IHN. Cell

culture, real-time RT-PCR and amplicon sequencing of a conventional PCR product are the recommended methods for confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis for all life stages (WOAH 2022b).

Other testing methods to detect IHNV include neutralising antibody test, indirect fluorescent antibody assay, enzyme-linked immunosorbent assay, in situ hybridisation assay and RT-PCR (Arakawa et al. 1990; Arnzen et al. 1991; Deering et al. 1991; Dhar et al. 2008; Dixon & Hill 1984; Jorgensen et al. 1991; LaPatra et al. 1989; WOA 2022b).

### 12.2.5 Treatment

There are no scientifically confirmed reports of effective treatments (Yong et al. 2019).

### 12.2.6 Control and prevention

Control methods for infection with IHNV are primarily aimed at preventing the introduction of the virus into susceptible populations. This is achieved through the implementation of biosecurity control policies and practices such as using RT-PCR for pre-screening of broodstock and discarding those that test positive for IHNV, using virus-free water supplies and treating water with UV or ozone (Meyers et al. 2003; Purcell et al. 2013; Winton 1991). The disinfection of eggs with iodophor is common practice although it is not always 100% effective (Bovo et al. 2005a; Goldes & Mead 1995). It was estimated that 5 out of 100 disinfected *O. mykiss* egg consignments would still lead to IHNV infections at receiving sites (Oidtmann et al. 2014).

Plasmid DNA vaccines containing the gene for the IHNV glycoprotein have proven highly efficacious against infection with IHNV resulting in the licensing of a DNA vaccine for commercial use in *S. salar* net pen aquaculture on the west coast of North America (Alonso & Leong 2013; Corbeil et al. 2000; Garver, LaPatra & Kurath 2005; Garver & Wade 2017) and in salmonids in Canada (Bondad-Reantaso et al. 2023). Several studies have investigated the use of natural and antiviral compounds to control IHNV infections but at present none are being actively used in aquaculture facilities (Balmer et al. 2017; Hasobe & Saneyoshi 1985; Hu et al. 2019; Li et al. 2019). Differences in IHNV susceptibility among salmonid populations and species have led to experimental trials of triploid or interspecies salmonid hybrids to develop aquaculture stock with greater resistance to IHNV to improve production (Barroso et al. 2008; LaPatra 1998; Purcell et al. 2010; Winton 1991).

If IHNV is detected on farms, there should be immediate destruction of the fish in affected ponds/tanks, rigorous disinfection and fallowing for a time before restocking (Meyers et al. 2003; Saksida 2006). IHNV is readily inactivated by common disinfectants with active ingredients such as sodium hypochlorite, iodophor, benzalkonium chloride, saponated cresol, formaldehyde and potassium permanganate solution (Bowker et al. 2016; Yoshimizu et al. 2005).

### 12.2.7 Impact of the disease

Economic losses from IHNV can be a direct consequence of fish mortality, or indirect such as from regulations restricting the movement of IHNV infected fish or the destruction of infected fish stocks to control the spread of the virus (Bootland & Leong 1999).

Two outbreaks of IHNV in Canada in 1992–1996 and 2001–2003 in *S. salar* net pens caused a combined estimated economic loss to the salmon industry of US\$40 million in animals representing US\$200 million in total lost sales (Garver et al. 2013a). The 2001–2003 outbreak resulted in 12 million

salmon either dying or being culled (Saksida 2006). An IHNV outbreak in *S. salar* net pens in Bainbridge Island, Washington, USA in 2012 led to 400,000 fish being destroyed or emergency harvested (NWIFC 2012).

### 12.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon or their reproductive material as import is not permitted.

There are biosecurity measures to manage the risk of IHNV in imported salmonid fish for human consumption (see [Appendix D](#)).

## 12.3 Risk assessment

Based on [chapter 4](#) and the technical information about IHNV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of IHNV meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 12.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that IHNV is present in all source countries.
- There are no reports of IHNV infecting live sturgeon by natural exposure. There is one report of IHNV infecting sturgeon experimentally by water exposure (LaPatra et al. 1995). It will be assumed that IHNV can infect live sturgeon.
- IHNV is expected to infect sturgeon life stages exported to Australia.
- Prevalence of IHNV in farmed salmonids can reach 100% and in wild populations can be up to 27%.
- There are no reports of IHNV associated with sturgeon reproductive material but it has been associated with reproductive material in other fish species (Bovo et al. 2005a).
- IHNV can survive in freshwater and seawater for extended periods.
- The viral load of IHNV in infected imported live sturgeon or their reproductive material is likely to be sufficient to cause infection in susceptible species.
- Inspection may detect sturgeon showing clinical signs of infection with IHNV and remove them before export. Sturgeon with mild or no clinical signs would be unlikely to be detected.
- Sturgeon reproductive material infected or contaminated with IHNV is unlikely to be detected during inspection because there would be no clinical signs.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of IHNV was estimated to be:

- Imported live sturgeon—**Low**.

- Imported sturgeon reproductive material—**Low**.

### 12.3.2 Exposure assessment

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

- IHNV can be transmitted horizontally via water or cohabitation and can remain infectious in water for an extended period.
- IHNV would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- Species susceptible to IHNV infection are present in Australia include salmonids and *Anguilla* species.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are susceptible to IHNV infection.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the upper end of the range (3–18°C) that IHNV causes mortality in salmonids (Bootland & Leong 1999).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infected sturgeon will be certain to be exposed to viable IHNV.
- Introduction into the wild may occur by direct release of imported live sturgeon, or associated waste, from the aquaculture facility into natural waters. This would be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for fish escapes and waste management that would exclude IHNV from discharges.
- Wild susceptible species would be less abundant than susceptible species in aquaculture facilities. Despite this, wild susceptible species would be expected to be exposed to IHNV released into natural waters due to the ability of the virus to survive independent of the host and susceptible species being present in Australian waters.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to IHNV in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

The partial likelihood of exposure to each exposure group to IHNV in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**Moderate**
- Wild susceptible species—**Low**.

### 12.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to IHNV in **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

The partial annual likelihood of entry and exposure of each exposure group to IHNV in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

### 12.3.4 Consequence assessment

#### Partial likelihood of establishment and spread (PLES)

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

- IHNV can be transmitted horizontally via water or cohabitation and can remain infectious in water for an extended period. Transmission between broodstock and progeny occurs.
- It is expected that susceptible species in contact with IHNV-infected fish would receive an infectious dose.
- Fish that survive IHNV infections may become carriers and sources of the virus.
- IHNV can infect salmonids and *Anguilla* species present in Australia.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are susceptible to IHNV infection.
- The likelihood of IHNV establishment, following a given quantity of IHNV entering the environment of an exposure group, is greatest for farmed susceptible species. This is due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- Live sturgeon or sturgeon reproductive material could be moved to other aquaculture facilities in Australia. Species polycultured with IHNV-infected sturgeon or sharing the same water, could also be moved to other facilities. It is expected that IHNV would establish in these facilities if present in the animals or reproductive material being translocated.
- Each state and territory have translocation protocols for aquaculture animals, which typically include consideration of IHNV.
- If IHNV were to establish on a farm it could spread to neighbouring farms and wild populations through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of IHNV be suspected and response measures initiated immediately. However, IHNV is effectively transmitted through water and can persist in the environment and on farms which share a common water source or equipment with an infected population.

- The likelihood of IHNV spread from farms to wild populations or neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however IHNV could spread this way. IHNV could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.
- If one or more index cases of IHNV 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 IHNV can survive in the environment, it could persist until susceptible hosts were to encounter it.
- The likelihood of IHNV in a wild population spreading to its natural geographic limits is greater than for other hazards with limited host ranges, for example, typical *Aeromonas salmonicida*, and would be more likely than for those hazards which cannot survive outside of a host for long periods. The ability of fish to be subclinically infected with IHNV and to remain carriers after surviving an infection would also aid its spread.
- If IHNV were to establish in the wild, especially in waters around aquaculture facilities, it may easily spread to farms through water intake due to IHNV being able to survive in the environment and being transmissible through water. 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 4, the partial likelihood of establishment and spread of IHNV in each exposure group for the outbreak scenario was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

### Determining adverse impacts resulting from the outbreak scenario

The factors considered relevant when determining the adverse impacts resulting from establishment and spread of IHNV were that:

#### *Direct effects*

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

- IHNV is primarily a disease of salmonids that can cause mortalities of up to 90% in populations. Production and productivity losses due to IHNV would be significant for the Australian salmonid industry with aquaculture production valued at approximately AU\$1 billion in 2020–21 (Tuyman & Dylewski 2022).
- Sturgeon are susceptible to IHNV and mortality has been associated with infection. The establishment of a sturgeon industry in Australia would be significantly affected by an outbreak of IHNV.
- IHNV may impact wild fisheries in Australia. There are reports of IHNV in wild fish and associated mortalities although no reports of associated declines in catch rates.
- Based on the impacts of IHNV infection on farming industries in Europe and the USA, IHNV 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*

- IHNV has a moderate host range and there are reports of IHNV infection in wild fish populations overseas. Although wild fish can also be subclinically infected or carriers of IHNV rather than clinically affected.
- The direct impact of IHNV 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 IHNV is listed as a notifiable disease by WOA 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 IHNV.
- If IHNV was confirmed at a 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 IHNV 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 IHNV from wild populations is unlikely to be undertaken.
- If a movement restriction area were put in place for an outbreak of IHNV, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- Eradication and control of IHNV 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 seafood suppliers, commercial wild catch fisheries and bait fisheries due to the host range of IHNV.
- 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.
- IHNV-infected fish may show clinical signs which would affect their marketability.
- IHNV 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 IHNV is a WOA-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to IHNV to avoid the possible introduction of IHNV.

- If IHNV were to become established, Australia could use zoning to maintain access to international markets for live susceptible species, including sturgeon and, if required, non-viable product.
- The impacts of IHNV 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*

- IHNV 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 IHNV.
- The impacts of IHNV 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 IHNV 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 IHNV outbreak would have an impact on communities such as causing loss of business and welfare concerns.
- The social impacts of IHNV establishment and spread are expected to be minor at the district or region level.

Table 19 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of IHNV. 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 19 Overall impact of establishment and spread of IHNV 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)	District or region	Minor	C

**Conclusion**

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

### 12.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for IHNV 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:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Moderate**.

### 12.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of IHNV from **imported live sturgeon** 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Very low**.

The partial annual risk of entry, establishment and spread of IHNV from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Very low**.

### 12.3.7 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 IHNV was found to be:

- Imported live sturgeon—**Moderate**.
- Imported sturgeon reproductive material—**Moderate**.

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

## 12.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for IHNV in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 12.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 20 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of IHNV but which on their own do not achieve Australia's ALOP for IHNV in imported live sturgeon or their reproductive material.

**Table 20 Biosecurity measures that on their own do not achieve Australia's ALOP for IHN**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Disease-free stock	<b>Yes:</b> Determination of IHN freedom would need to be to a standard consistent with that recommended by the World Organisation for Animal Health (WOAH), or equivalent.
2	Post-arrival quarantine (PAQ)	<b>Yes:</b> The stress of transport can induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
3	Post-arrival batch testing	<b>Yes:</b> Cell culture, real-time RT-PCR and amplicon sequencing of a conventional PCR product methods can detect IHN (WOAH 2022b). Under this scenario, testing is conducted under departmental control and oversight.

### 12.4.2 Biosecurity measures that in combination achieve Australia's ALOP

A combination of biosecurity measures 1, 2 and 3 when applied to **imported live sturgeon** would reduce the likelihood of entry of IHN from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1, 2 and 3 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of IHN from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

# 13 Nervous necrosis virus

## 13.1 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 (Oliveira 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 2022a) 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 WOAHA due to the widespread distribution of NNV (OIE 2021c). However, VER is on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). 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.

## 13.2 Technical information

### 13.2.1 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, Oliveira & 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, Oliveira & 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).

### 13.2.2 Epidemiology

#### Host range

Species which are susceptible to infection with BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV and TPNNV (N= natural exposure; E= experimental exposure) include but are not limited to:

- *Dicentrarchus labrax*<sup>N,E</sup> (European seabass) – BFNNV, SJNNV, RGNNV/SJNNV (Athanassopoulou et al. 2003; Cutrín et al. 2007; Thiery, Cozien & de Boisseson 2004)
- *Gadus morhua*<sup>N</sup> (Atlantic cod) – BFNNV (Johnson et al. 2002; Nylund et al. 2008)
- *Gadus macrocephalus*<sup>N</sup> (Pacific cod) – BFNNV (Mao et al. 2015)
- *Hippoglossus hippoglossus*<sup>N</sup> (Atlantic halibut) – BFNNV (Grotmol et al. 1997; Starkey et al. 2000)
- *Melanogrammus aeglefinus*<sup>N</sup> (haddock) – BFNNV (Gagne et al. 2004)
- *Paralichthys olivaceus*<sup>N</sup> (Japanese flounder) – BFNNV, TPNNV (Iwamoto et al. 2000; Nishizawa et al. 1997)
- *Pseudopleuronectes americanus*<sup>N</sup> (winter flounder) – BFNNV (Barker et al. 2002)
- *Pseudocaranx dentex*<sup>N</sup> (striped jack, silver trevally, white trevally) – SJNNV (Mori et al. 1992)
- *Salmo salar*<sup>E</sup> (Atlantic salmon) – BFNNV (Korsnes et al. 2005)
- *Scophthalmus maximus*<sup>N</sup> (turbot) – BFNNV (Bloch, Gravningen & Larsen 1991)

- *Solea senegalensis*<sup>N</sup> (Senegalese sole) – SJNNV, RGNNV/SJNNV (Cutrín et al. 2007; Oliveira et al. 2009; Thiery, Cozien & de Boisseson 2004)
- *Solea solea*<sup>N</sup> (common sole) – BVNNV (Starkey et al. 2001)
- *Sparus aurata*<sup>N</sup> (gilthead seabream) – SJNNV/RGNNV, RGNNV/SJNNV (Cutrín et al. 2007; Oliveira et al. 2009; Toffan et al. 2017; Volpe et al. 2020)
- *Takifugu rubripes*<sup>N</sup> (tiger puffer) – TPNNV (Nakai et al. 1994; Nishizawa et al. 1997)
- *Trachurus japonicus*<sup>N, E</sup> (Japanese jack mackerel) – SJNNV (Nishioka et al. 2016)
- *Verasper moseri*<sup>N</sup> (Barfin flounder) – BFNNV (Muroga 1995).

Species for which PCR-positive results have been reported but no active infection has been demonstrated (N= natural exposure; E= experimental exposure) include:

- *Anguilla anguilla*<sup>N</sup> (European eel) – SJNNV (Bandín et al. 2014)
- *Argyrosomus regius*<sup>N</sup> (meagre) – SJNNV (Lopez-Jimena et al. 2010)
- *Carangoides equula*<sup>N</sup> (whitefin trevally) – SJNNV (Sakamoto et al. 2008)
- *Ctenolabrus rupestri*<sup>N</sup> (goldsinny wrasse) – BFNNV (Korsnes et al. 2017)
- *Etrumeus teres*<sup>N</sup> (round herring) – BFNNV (Sakamoto et al. 2008)
- *Evynnis cardinalis*<sup>N</sup> (threadfin porgy) – SJNNV (Ma et al. 2015)
- *Labrus bergylta*<sup>N</sup> (ballan wrasse) – BFNNV (Korsnes et al. 2017)
- *Pagrus major*<sup>N</sup> (red seabream) – SJNNV (Sakamoto et al. 2008)
- *Scomber japonicus*<sup>N</sup> (chub mackerel) – SJNNV (Sakamoto et al. 2008)
- *Seriola dumerili*<sup>N</sup> (greater amberjack, amberjack) – SJNNV (Sakamoto et al. 2008)
- *Seriola quinqueradiata*<sup>N</sup> (Japanese amberjack) – SJNNV (Sakamoto et al. 2008)
- *Solea solea* (common sole, dover sole)<sup>N</sup> – RGNNV/SJNNV (Panzarin et al. 2012)
- *Symphodus melops*<sup>N</sup> (corking wrasse) – BFNNV (Korsnes et al. 2017)
- *Trachurus mediterraneus*<sup>N</sup> (Mediterranean horse mackerel) – RGNNV/SJNNV (Bitchava et al. 2019).

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 is only one report of NNV infecting sturgeon. An NNV infection causing morbidity and mortality was reported in *Acipenser gueldenstaedtii* (Russian sturgeon) from a freshwater farm in Greece that also contained NNV-infected *D. labrax* (Athanasopoulou, Billinis & Prapas 2004). A follow-up study identified the NNV in the sturgeon as most similar to a RGNNV isolated from *D. labrax* from marine cages in the region (Xylouri et al. 2007). There have been no reports of BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV or TPNNV infecting sturgeon.

### 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 (Athanasopoulou et al. 2003), whereas the opposite form, RGNNV/SJNNV, is widespread in Southern Europe including in Croatia, Cyprus, Greece, Italy, Portugal and Spain (Oliveira et al. 2009; Panzarin et al. 2012).

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

### Prevalence

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 *T. japonicus* 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 *A. regius* caught in the Iberian Peninsula (Lopez-Jimena et al. 2010). A survey of wild *A. anguilla* 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 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; WOA 2019).

SJNNV-positive *P. dentex* 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 *P. dentex* in a fish farm in Japan (Arimoto et al. 1993). SJNNV infecting farmed *S. senegalensis* 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 *H. hippoglossus* due to BFNNV infections (Grotmol et al. 1997; Johansen et al. 2004; Johansen et al. 2002). Farmed *D. labrax*, in both recirculating and fresh open water production facilities in Greece, suffered NNV outbreaks in 2000 reaching mortalities of 30% (Athanasopoulou et al. 2003). Outbreaks of BFNNV in farmed *G. morhua* in the UK in 2000 resulted



in mortalities of approximately 2% over a 3-month period (Starkey et al. 2001). BFNNV infection in *S. solea* 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 *G. morhua* 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 *G. macrocephalus* larvae-rearing facility in China (Mao et al. 2015).

In 2014, *S. aurata* 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 *S. aurata* farm in Europe caused a cumulative mortality of 60% (Toffan et al. 2017). In an Italian hatchery, multiple RGNNV/SJNNV outbreaks during 2017–2018 in *S. aurata* and *D. labrax* 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, Oliveira & Bandin 2015; WOA 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; Mushiakhe 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, Mushiakhe & Arimoto 1998; Mushiakhe 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 *P. dentex* suggest that larvae are primarily infected with the virus through broodstock showing no clinical signs (Arimoto et al. 1992; Mori, Mushiakhe & Arimoto 1998; Mushiakhe et al. 1994; Nguyen et al. 1997; Nguyen, Nakai & Muroga 1996). Following a natural infection, infectious RGNNV/SJNNV could still be detected in survivor *S. aurata* 6–7 months later (Toffan et al. 2017). Similarly, BFNNV could be re-isolated from *H. hippoglossus* 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; Mushiakhe et al. 1994; Souto, Oliveira & 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* (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 *A. salina* was capable of transmitting RGNNV/SJNNV to *S. senegalensis* larvae (via feeding), that resulted in clinical signs and high mortality (Vazquez-Salgado et al. 2020).

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 *P. dentex* and *T. japonicus* exposed to  $10^7$  TCID<sub>50</sub>/mL SJNNV for 1 hour resulted in 90–100% mortality at 3–9 days post infection (dpi) (Nishioka et al. 2016). Injection of *P. dentex* (mean weight 84 g) with  $5 \times 10^5$  TCID<sub>50</sub>/mL SJNNV induced an infection but did not cause any clinical signs or mortality in the fish (Banu et al. 2007). *S. senegalensis* juveniles (mean weight 2 g) infected by bath exposure for 3 hours using  $10^5$  TCID<sub>50</sub>/mL SJNNV or RGNNV/SJNNV started dying from 5 dpi and reached 100% mortality at 18–25 dpi (Souto et al. 2015). *D. labrax* (mean weight 0.2 g) challenged by bath exposure to  $10^4$  TCID<sub>50</sub>/mL SJNNV, RGNNV/SJNNV and SJNNV/RGNNV strains for 2 hours resulted in clinical signs of infection and mortality (Vendramin et al. 2014).

## 13.2.3 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<sub>50</sub>/g for *P. dentex* and  $10^{4.0}$ – $10^{7.6}$  TCID<sub>50</sub>/g for *T. japonicus* (Nishioka et al. 2016). SJNNV intramuscularly injected into *P. dentex* (mean weight 84 g) reached a maximum titre of  $10^{7.9}$  TCID<sub>50</sub>/g in the spinal cord at 5 dpi,  $10^{6.9}$  TCID<sub>50</sub>/g in the brain at 7 dpi,  $10^{5.1}$  TCID<sub>50</sub>/g in the eye at 14 dpi and  $10^{8.2}$  TCID<sub>50</sub>/g in the kidney at 5 dpi (Banu et al. 2007). In bath challenge experiments of *D. labrax* (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  $2.4 \times 10^8$  RNA copies/g and  $1.6 \times 10^7$  TCID<sub>50</sub>/g (Souto et al. 2015).

Immersion challenge of *S. senegalensis* juveniles (mean weight 1 g) with SJNNV and RGNNV/SJNNV resulted in virus titres being detected in the brain at 1 dpi with  $1.1 \times 10^4$  and  $9.4 \times 10^4$  RNA copies/g, respectively, that reached  $2.8 \times 10^8$  and  $2.4 \times 10^9$  RNA copies/g, respectively, by 15 dpi. The SJNNV and RGNNV/SJNNV infective viral titres in the brain at 1–2 dpi were  $1.7 \times 10^2$  and  $2.4 \times 10^2$  TCID<sub>50</sub>/g, respectively and by 15 dpi, had increased to  $5.6 \times 10^6$  and  $5.6 \times 10^7$  TCID<sub>50</sub>/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  $1.3 \times 10^6$  copies/g in the skin at 12 dpi,  $2.8 \times 10^3$  copies/g in fins at 14 dpi,  $1.2 \times 10^4$  copies/g in gills at 15 dpi and  $1.0 \times 10^3$  copies/g in intestines at 1 dpi. For RGNNV/SJNNV, the highest RNA copy numbers were  $4.1 \times 10^5$  copies/g in skin at 12 dpi,  $1.6 \times 10^5$  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 *S. senegalensis* (mean weight 2 g), the viral titres in dead fish was highest at  $6.9 \times 10^5$  TCID<sub>50</sub>/g at 10–13 dpi for fish infected with RGNNV/SJNNV and at  $7.4 \times 10^4$  TCID<sub>50</sub>/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 *G. macrocephalus* showing clinical signs (Mao et al. 2015). The BFNNV copy number in *G. macrocephalus* eggs ranged from  $<10$ – $10^3$  copies/ng total RNA (Mao et al. 2015).

### 13.2.4 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).

*A. gueldenstaedtii* (Russian sturgeon) infected with RGNNV displayed lethargy, abnormal swimming and lack of appetite (Athanassopoulou, Billinis & Prapas 2004).

#### 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).

In RGNNV-infected *A. gueldenstaedtii*, necrosis and vacuolation was only detected in the brain and spinal cord (Athanassopoulou, Billinis & Prapas 2004).

#### Testing

In infected farms, the probability of detecting NNV is normally higher in juveniles than in older fish, while during spawning season the virus may be found in the ovaries and testes of broodstock (Dalla Valle et al. 2000; Mushiakhe et al. 1994).

Isolation of viral activity 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).

### 13.2.5 Treatment

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

### 13.2.6 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, avoidance of NNV-infected fish for feeding and cleaning, 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 *D. labrax* 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).

### 13.2.7 Impact of the disease

NNV causes a devastating disease among cultured marine fish worldwide and results in huge 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 an indirect but significant cost loss which is often underestimated (Vendramin et al. 2014). 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 *S. aurata* and *D. labrax* where production in 2020 was 208 tonnes and 199,476 tonnes, respectively, in Mediterranean countries (EURL for Fish and Crustacean Diseases 2021).

### 13.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon and their reproductive material as import is not permitted.

## 13.3 Risk assessment

Based on [chapter 4](#) and the technical information about NNV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of NNV meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 13.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that NNV is present in all source countries.
- Whereas RGNNV infects a wide range of fish species and is present in Australia, BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV or TPNNV are exotic and infect a narrower range of species (Bandín & Souto 2020; Munday, Kwang & Moody 2002).
- There are no reports of BFNNV, SJNNV, SJNNV/RGNNV, RGNNV/SJNNV or TPNNV infecting live sturgeon either by natural or experimental exposure.
- There is one report of RGNNV infecting *A. gueldenstaedtii* in a freshwater farm in Greece that caused morbidity and mortality (Athanasopoulou, Billinis & Prapas 2004). RGNNV is present in Australia.
- The prevalence of NNV can be up to 100% in farmed fish populations and 48% in wild fish.
- There are no reports of NNV associated with sturgeon reproductive material. However, NNV has been detected in the reproductive material of several other fish species.
- 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.
- Inspection may detect sturgeon showing clinical signs that are typical of infection with NNV and remove them before export. Sturgeon with mild or no clinical signs would not be identified through visual inspection.
- Sturgeon reproductive material infected or contaminated with NNV are unlikely to be detected during inspection because there would be no clinical signs.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of NNV was estimated to be:

- Imported live sturgeon—**Negligible**.
- Imported sturgeon reproductive material—**Negligible**.

As the entry assessment of NNV was negligible for live sturgeon and their reproductive material, which achieves Australia's ALOP, the risk assessment was not continued.

# 14 Polypodium hydriforme

## 14.1 Background

*Polypodium hydriforme* is an obligate cnidarian endoparasite of Acipenserid and polyodontid fishes. It can impact caviar production and reduce recruitment rates. Infection with *P. hydriforme* was first described in the eggs of *Acipenser ruthenus* (sterlet sturgeon) from the Volga River, the Russian Federation, in 1871 ((Owsjannikow 1871) cited in (Raikova 1994)). Since that time, it has been reported in sturgeon and paddlefishes in Eurasia and North America (Dadswell et al. 1984; Dick, Holloway & Choudhury 1991; Raikova 2002).

Infection with *P. hydriforme* is not listed as notifiable to the World Organisation for Animal Health (WOAH) (OIE 2020), and is not included on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). *P. hydriforme* is considered exotic to Australia.

## 14.2 Technical information

### 14.2.1 Agent properties

*P. hydriforme* is the sole occupant of the Polypodiozoa class (Evans et al. 2008) and phylogenomic evidence places it as a sister to the Myxozoa, a diverse clade of endoparasitic cnidarians (Chang et al. 2015; Kayal et al. 2018). In contrast to other myxozoans, *P. hydriforme* does not have a degenerate body form. Instead, it displays cnidarian-like features including tentacles, a gut and a mouth (Chang et al. 2015; Raikova 1994; Raikova, Suppes & Hoffman 1979).

The life cycle of *P. hydriforme* consists of a parasitic larval stage and a free-living, actively feeding adult stage (Raikova 1994). Most of its life cycle (several years), including all embryonic stages, are spent in developing eggs of female acipenseriform fish, where it lies dormant as a binucleate cell (Raikova, Suppes & Hoffman 1979). Just before the spawning of the host fish, the *P. hydriforme* stolon everts and all the yolk of the infected oocyte is transferred into the gastral cavity of the everted stolon. The parasite is then released into the freshwater from the infected eggs of the host fish after spawning (Raikova 1994; Raikova & Raikova 2016). In the water, the everted stolon immediately fragments into free-living tentacled individuals. Each individual forms a mouth at the place of former attachment to the stolon and begins to feed on small freshwater invertebrates. Free-living individuals have been observed over a couple of months (Okamura et al. 2020). These individuals multiply by longitudinal fission and later, during the summer, produce gonads that, packed in a gonadophore and equipped with polar bodies, are released into the water (Raikova 1994). After release of the gonadophore, the free-living *P. hydriforme* die (Raikova 1994; Raikova & Raikova 2016). The gonadophores adhere to a new female fish host, enabling *P. hydriforme* to infect the fish and start its life cycle again (Raikova 1994). It is still unclear how *P. hydriforme* infects the female fish (Raikova, Suppes & Hoffman 1979).

### 14.2.2 Epidemiology

#### Host range

Species which are reported to be susceptible to infection with *P. hydriforme* (N= natural exposure; E= experimental exposure) include but are not limited to:

- *Acipenser baerii* <sup>N</sup> (Siberian sturgeon) ((Pronin 1975)) cited in (Raikova 2002))

- *Acipenser brevirostrum*<sup>N</sup> (shortnose sturgeon) (Raikova 2002)
- *Acipenser fulvescens*<sup>N</sup> (lake sturgeon) (Dick, Holloway & Choudhury 1991)
- *Acipenser gueldenstaedtii*<sup>N</sup> (Russian sturgeon) ((Dogiel 1940) cited in (Raikova 2002))
- *Acipenser medirostris*<sup>N</sup> (green sturgeon) ((Artuchin & Andronov 1989) cited in (Raikova 2002))
- *Acipenser nudiiventris*<sup>N</sup> (ship sturgeon) ((Trussov 1947) cited in (Raikova 2002))
- *Acipenser ruthenus*<sup>N</sup> (sterlet sturgeon) ((Owsjannikow 1871) cited in (Raikova 2002))
- *Acipenser schrenckii*<sup>N</sup> (amur sturgeon) ((Svirsky) cited in (Raikova 2002))
- *Acipenser stellatus*<sup>N</sup> (stellate sturgeon) ((Dogiel 1940) cited in (Raikova 2002))
- *Acipenser transmontanus*<sup>N</sup> (white sturgeon) (Dick, Holloway & Choudhury 1991)
- *Huso dauricus*<sup>N</sup> (kaluga sturgeon) ((Svirsky 1984) cited in (Koshelev, Ruban & Shmigirilov 2014))
- *Huso huso*<sup>N</sup> (beluga) ((Svirsky 1984) cited in (Raikova 2002))
- *H. huso* × *A. ruthenus*<sup>N</sup> (bester) (I.A. Burtsev (pers. comm.) cited in (Raikova 2002))
- *Polyodon spathula*<sup>N</sup> (paddlefish) (Suppes & Meyer 1975)
- *Scaphirhynchus platyrhynchus*<sup>N</sup> (shovelnose sturgeon) (Evans et al. 2008).

The severity of infection and the percentage of eggs infected in an individual host varies. For example, *A. ruthenus* is reported to be the most extensively affected species with up to 100% of eggs infected (Raikova 1994) whereas the percentage of wild *P. spathula* eggs infected with *P. hydriforme* ranged from 0.04–0.37% (Suppes & Meyer 1975).

### Geographical distribution

Infection with *P. hydriforme* was first described in the Russian Federation ((Owsjannikow 1871) cited in (Raikova 1994)) and has since been detected in Iran, Kazakhstan, the Republic of Moldova and Romania (Raikova 2002). It has also been reported in Canada (Dick, Holloway & Choudhury 1991) and the United States of America (USA) (Dadswell et al. 1984; Suppes & Meyer 1975).

### Prevalence

During a parasitological survey of natural waters in Hungary during 1985–1992, 31.9% (n=69) of *A. ruthenus* spawners were found to be infected with *P. hydriforme* (Baska 1999). In wild *A. fulvescens* sampled from two provinces in Canada, 100% of females with 3 mm and larger eggs were infected with *Polypodium* sp., however, *P. hydriforme* was only detected in 4.5% (n=88) of all females (Dick, Holloway & Choudhury 1991). The prevalence of *P. hydriforme* in wild *P. spathula* sampled in 2017 and 2018 in Oklahoma, USA was 49.2% (n=193) and 45.2% (n=62), respectively (Okamura et al. 2020). The percentage of eggs infected with *P. hydriforme* was not determined in this study, but was not considered to be significant (Okamura et al. 2020). *P. hydriforme* has also been detected in wild *P. spathula* from the Missouri River, USA at a prevalence of 41.2% (n=24) with less than 1% of the eggs in the paddlefish infected (Holloway, Dick & Ottinger 1991). The parasite was detected in 18% (n=11) of wild adult *S. platyrhynchus* sampled in 2008 in Indiana, USA (Sepúlveda, Stefanavage & Goforth 2010). Spawning fish from the Amur River in the Russian Federation were sampled across summer to autumn of 2005–2009 and 55.9% (n=102) of *H. dauricus* and 57.1% (n=84) of *A. schrenckii* were found to be infected with *P. hydriforme* (Koshelev, Ruban & Shmigirilov 2014).

Comparatively, *P. hydriforme* prevalence in *H. dauricus* from the Amur River has also been reported to range from 42.8% ((Svirsky 1984) cited in (Koshelev, Ruban & Shmigirilov 2014)) to 100% ((Yukhimenko & Belyaev 2002) cited in (Koshelev, Ruban & Shmigirilov 2014)).

No reports were found on the prevalence of *P. hydriforme* in farmed sturgeon.

#### **Mortalities**

No reports of mortalities due to *P. hydriforme* infection were found.

#### **Transmission**

The infection process is poorly understood and has only been observed for *A. ruthenus*. The free-living tentaculate stages of *P. hydriforme* are believed to remain in the vicinity of the spawned fish eggs. Therefore, in some cases, infection of fish following hatching on common spawning grounds is likely to occur (Okamura et al. 2020). Attempts to infect *A. ruthenus* with free-living cultured *P. hydriforme* in the laboratory were unsuccessful (Raikova 1994). The *P. hydriforme* individuals did not attach to any part of the fish and were not swallowed (Raikova 1994). It is suggested that infection of acipenserids occurs during their early life as pre-larval fish when their skin is more delicate and accessible to gametophores of *P. hydriforme* (Raikova 1994).

It is considered that *P. hydriforme* is introduced into some rivers with the transfer of acipenserids (Raikova 2002). It was also suggested that whilst it is possible for *P. hydriforme* to be imported through the movement of eggs, it is of minimal risk and a relatively rare event (Bauer 1991).

#### **Infectious dose**

The dose of *P. hydriforme* required to infect acipenserids is unknown. Attempts to infect *A. ruthenus* with free-living cultured *P. hydriforme* in the laboratory were unsuccessful (Raikova 1994) and the exact mechanism by which fish become infected is unknown.

### **14.2.3 Pathogenesis**

#### **Tissue tropism**

*P. hydriforme* is restricted to the eggs of female susceptible species.

#### **Tissue titre**

No reports of tissue titre of *P. hydriforme* were found. Usually only one parasite occurs in an egg, but it has been reported that two parasites have been found in a single egg (Raikova 2002)((Raikova 1987) cited in (Raikova 1994)).

### **14.2.4 Diagnosis**

#### **Clinical signs**

During the later stages of development, infected eggs are visually different from uninfected eggs. They become marbled, white to ash grey in colour and enlarged compared to normal eggs. The diameter of infected eggs is reported to be over 3.5 times the size of an uninfected egg and up to 42 times the volume (Dick, Holloway & Choudhury 1991; Raikova 1994, 2002; Raikova, Suppes & Hoffman 1979). The larger size of infected eggs is reportedly due to increased intake of water by those eggs (Judd, Tripp & Herzog 2022).

#### **Pathology**

Differences in the nutritional content of infected and uninfected eggs were found (Judd, Tripp & Herzog 2022). It was suggested that most of the nutritional differences were due to the *P. hydriforme*



digesting the egg contents as it was developing and the nutritional content of the *P. hydriforme* themselves (Judd, Tripp & Herzog 2022). No residual effects on the nutrient levels in the uninfected eggs were found and it was hypothesised that a low-level infection of *P. hydriforme* would have little effect on the development of larvae in uninfected eggs (Judd, Tripp & Herzog 2022). However, it was suggested that high infection levels may affect overall fecundity of female fish (Judd, Tripp & Herzog 2022). Abnormal ovaries have been reported in *A. fulvescens* with high (20%) levels of *P. hydriforme* infection although studies directly assessing this have not been undertaken (Hoffman, Raikova & Yoder 1974).

Infection of *P. spathula* with *P. hydriforme* was linked to decreases in roe fat weight independent of fish length, weight, age or roe weight. It was concluded that infection therefore diminishes *P. spathula* energy reserves which could cause decreases in host fitness (Okamura et al. 2020).

### Testing

Detection of *P. hydriforme* is typically done through examination of the eggs of female fish using microscopy and the subsequent morphological identification of the parasite. Infection may be obvious using the naked eye, however, if the eggs are not mature, there is the potential that *P. hydriforme* may be missed (Dick, Holloway & Choudhury 1991).

Molecular techniques have been designed to identify *P. hydriforme* stolons from the free-living stage (Siddall et al. 1995) but no commercial diagnostic methods are available.

### 14.2.5 Treatment

No reports about treatment of infection with *P. hydriforme* were found.

### 14.2.6 Control and prevention

No reports of control measures for *P. hydriforme* were found. This is because infection with *P. hydriforme* occurs primarily in the wild. It has been suggested that the risk of infection of larval fish can be reduced if sturgeon are displaced from spawning grounds. This is because some sturgeon seek refuge in the spawning substrate during daylight, putting them at increased risk of interacting with the free-living tentaculate stages of *P. hydriforme* and becoming infected (Okamura et al. 2020). Removing those fish from the spawning substrate may reduce the likelihood of their infection. It is expected that control of *P. hydriforme* in an aquaculture facility could occur through good biosecurity practices and separation of newly hatched fish from contaminated water. However, prevention of infection would be unknown until fish are reproductively mature, a typically long period for sturgeons and paddlefishes (Okamura et al. 2020).

### 14.2.7 Impact of the disease

Impacts of infection with *P. hydriforme* are varied and depend upon the prevalence and intensity of the infection. If a high percentage of females are infected and a high proportion of the eggs of individual fish are parasitized, then the reproductive potential of the population could be compromised as could the quality of the caviar.

*P. hydriforme*-infected eggs were not considered to be a significant concern for *P. spathula* caviar quality in north-eastern Oklahoma in 2017–2018 (Okamura et al. 2020). This contrasts with impacts on caviar in the Russian Federation where 100% and 25% of eggs were reported as infected in *A. ruthenus* and *A. gueldenstaedtii*, respectively, leading to production losses (Raikova 2002).

It has been reported that *P. hydriforme* was responsible for reducing the reproductive capacity of *H. dauricus* and *A. schrenckii* which caused serious damage to the sturgeon fishery in the Amur River in the Russian Federation ((Svirsky 1984) cited in (Koshelev, Ruban & Shmigirilov 2014)). However, infection with *P. hydriforme* did not impact the reproductive success of *A. fulvescens* in the Saint Clair River, USA (Thomas & Muzzall 2009). The prolonged high densities and close quarters experienced by sturgeon in aquaculture (from eggs in hatching jars to fingerlings in raceways) may promote infection of larvae, and this could ultimately contribute to fish population declines if aquaculture is used for restoration stocking purposes (Okamura et al. 2020).

#### 14.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon or their reproductive material as import is not permitted.

### 14.3 Risk assessment

Based on [chapter 4](#) and the technical information about *P. hydriforme* presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of *P. hydriforme* meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

#### 14.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that *P. hydriforme* is present in all source countries.
- Infection with *P. hydriforme* occurs only in acipenserid and polyodontid fish.
- *P. hydriforme* is expected to infect sturgeon life stages that would be exported to Australia.
- *P. hydriforme* infects sturgeon eggs but not milt.
- The prevalence of *P. hydriforme* in farmed sturgeon is unknown. Prevalence in wild sturgeon can range from <1–100%.
- The load of *P. hydriforme* in infected imported live sturgeon or their reproductive material is likely to be sufficient to cause infection in susceptible species.
- The free-living stage of the parasite can survive independently of the fish host.
- It is unknown how long *P. hydriforme* can survive in harvested sturgeon eggs.
- Inspection of live sturgeon will not detect infection with *P. hydriforme* as there are no clinical signs of disease in live animals. Visual inspection of eggs will likely detect infection if at high prevalence and late developmental stage.

#### Conclusion

Based on this information and using the qualitative likelihood descriptor in Table 4, the annual likelihood of entry of *P. hydriforme* was estimated to be:

- Imported live sturgeon—**High**.

- Imported sturgeon reproductive material—**High**.

### 14.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for *P. hydriforme* were that:

- Infection with *P. hydriforme* is believed to occur only in pre-larval fish. Once the fish has been invaded, *P. hydriforme* remains dormant until the fish eggs mature.
- Any viable *P. hydriforme* which enter the environment would be capable of persisting as free-living parasites for an extended period.
- *P. hydriforme* would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species.
- *P. hydriforme* is not known to infect species other than acipenserid and polyodontid fish. Aside from those species being considered for import in this BIRA, no other known susceptible species are present in Australia.
- Aquaculture species most likely to be polycultured with imported sturgeon such as trout are not susceptible to *P. hydriforme*.
- Culture conditions in aquaculture facilities (e.g. high stocking densities) would typically mean that any farmed susceptible species grown with or sharing the same water as infected sturgeon will be certain to be exposed to viable *P. hydriforme*.
- Introduction into the wild may occur by direct release of imported live sturgeon or its associated wastes into natural waters. Although this is considered a low likelihood for direct exposure due to the regulations that aquaculture facilities must operate under.
- Native Australian species are not known to be susceptible to infection with *P. hydriforme* and there are no wild populations of sturgeon species present.
- It is unknown if the environmental conditions of Australia are optimal for *P. hydriforme*.

#### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to *P. hydriforme* in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Negligible**.

The partial likelihood of exposure of each exposure group to *P. hydriforme* in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Negligible**.

### 14.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to *P. hydriforme* in **imported live sturgeon** 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Negligible**.

The partial annual likelihood of entry and exposure of each exposure group to *P. hydriforme* in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species— **Moderate**.
- Wild susceptible species—**Negligible**.

### 14.3.4 Consequence assessment

#### Partial likelihood of establishment and spread

The key points considered relevant when determining the partial likelihood of establishment and spread of *P. hydriforme* were that:

- Infection with *P. hydriforme* is believed to occur only in pre-larval fish. Once the fish has been invaded, *P. hydriforme* remains dormant until the fish eggs mature.
- *P. hydriforme* can persist for an extended period in the environment following release from spawned eggs.
- *P. hydriforme* only infects acipenserid and polyodontid fish.
- Non-sturgeon species polycultured with sturgeon are not known to be susceptible to infection with *P. hydriforme*.
- Live sturgeon or their reproductive material could be moved to other facilities in Australia for grow out. It is possible that *P. hydriforme* would establish in these facilities.
- If *P. hydriforme* was to occur in a farm, it is not expected to spread and establish in wild populations surrounding the farm. This is because no Australian native species are susceptible to infection with *P. hydriforme* and there are no wild populations of sturgeon present.

#### Conclusion

Based on this information and using the descriptors in Table 4, the partial likelihood of establishment and spread of *P. hydriforme* in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Negligible**.

#### Determining adverse impacts resulting from the outbreak scenario

The factors considered relevant when determining the adverse impacts resulting from establishment and spread of *P. hydriforme* were that:

##### *Direct effects*

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

- *P. hydriforme* is only known to infect species of sturgeon. Therefore, it is not expected to affect any other species of farmed fish in Australia, but the establishment of a sturgeon industry in Australia would be affected by an outbreak of *P. hydriforme*.
- *P. hydriforme* does not cause mortality in infected sturgeon but could cause a reduction in caviar production. Up to 100% of eggs can be infected with *P. hydriforme*.
- There are no known wild species of susceptible animals in Australia. Therefore, it is expected that there would be no impact on Australia's wild fisheries due to an outbreak of *P. hydriforme*.
- Based on the impact of *P. hydriforme* in Eurasia and North America, the establishment and spread of *P. hydriforme* in Australia would be expected to cause minor impacts at the state or territory 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*

- *P. hydriforme* is only known to infect sturgeon and there are no wild populations present in Australia. Therefore, it is expected that *P. hydriforme* would not establish in the environment.
- The direct impact of *P. hydriforme* on the living environment is not expected to be discernible at any level.

*Indirect effects*

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

- Infection with *P. hydriforme* is not listed as a notifiable disease by WOA and is not included on Australia's *National list of reportable diseases of aquatic animals*. Although not listed in Australia, state and territory governments would be expected to report on the presence of an unlisted agent that has never been reported in Australia.
- If *P. hydriforme* was confined to a farm, then attempts at eradication may be undertaken.
- Eradication and control of *P. hydriforme* is expected to cause minor impacts at the state or territory 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*

- 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.
- If *P. hydriforme* were to become established in an aquaculture facility it would be expected to affect caviar production.
- *P. hydriforme* establishment and spread would likely have a minor impact at the local 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 *P. hydriforme* is not a WOAHA-listed disease. There is no information available suggesting that importing countries would have import requirements or closed borders to the import of live sturgeon to avoid the introduction of *P. hydriforme*.
- The impacts of *P. hydriforme* establishment and spread on international trade are not expected to be discernible at any level.

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

- *P. hydriforme* are only known to infect sturgeon and there are no known susceptible species in the wild.
- The impact of *P. hydriforme* establishment and spread on the biodiversity of the environment 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*

- In local areas where a sturgeon aquaculture industry is established, *P. hydriforme* outbreaks could cause loss of business and welfare concerns.
- The social impacts of *P. hydriforme* establishment and spread are expected to be minor at the local level.

Table 21 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of *P. hydriforme*. 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 21 Overall impact of establishment and spread of *P. hydriforme* for the outbreak scenario**

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	State or territory	Minor	D
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	State or territory	Minor	D
	Economic (domestic trade effects and impact on other associated industries)	Local	Minor	B
	Economic (international trade effects)	Local	Unlikely to be discernible	A
	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)	Local	Minor	B

**Conclusion**

The overall impact of establishment and spread of *P. hydriforme* was estimated to be **low**.

### 14.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for *P. hydriforme* 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Negligible**.

### 14.3.6 Determination of partial annual risk

The partial annual risk of entry, establishment and spread of *P. hydriforme* from **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Negligible**.

The partial annual risk of entry, establishment and spread of *P. hydriforme* from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Negligible**.

### 14.3.7 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 *P. hydriforme* was found to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

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

## 14.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for *P. hydriforme* in imported live sturgeon or their eggs to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 14.4.1 Biosecurity measures that on their own achieve Australia's ALOP

#### Sourcing from disease-free stocks

When determining if sourcing live sturgeon or their eggs from a stock recognised by the department as free of *P. hydriforme* would reduce the likelihood of entry of *P. hydriforme*, the factors considered were:

- *P. hydriforme* been detected in Eurasia and North America (Dadswell et al. 1984; Dick, Holloway & Choudhury 1991; Raikova 2002).

- *P. hydriforme* freedom would need to be assessed to a standard consistent with that recommended for World Organisation for Animal Health (WOAH) listed diseases, or equivalent.

Sourcing from disease-free stocks alone would be sufficient to reduce the likelihood of entry of *P. hydriforme* in **imported live sturgeon** or their **eggs** from **high** to **very low**. This reduces the overall annual restricted risk of live sturgeon or their eggs to **negligible**, thereby achieving Australia's ALOP.

#### 14.4.2 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 22 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of *P. hydriforme* but which on their own or in combination do not achieve Australia's ALOP for *P. hydriforme* in imported live sturgeon or their eggs.

**Table 22 Biosecurity measures that on their own do not achieve Australia's ALOP for *P. hydriforme***

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Pre-export parasite treatment	<b>No:</b> <i>P. hydriforme</i> is an internal parasite. Parasite treatment that is externally applied to sturgeon will not be effective and is not considered further.
2	Post-arrival quarantine (PAQ)	<b>No:</b> Infected sturgeon do not show clinical signs so culturing for a PAQ period will not induce a clinical infection.
3	Post-arrival batch testing	<b>No:</b> <i>P. hydriforme</i> is only present inside the eggs which would be difficult to source for testing. Molecular techniques exist to identify stolons from the free-living stage (Siddall et al. 1995) but there is no diagnostic test available.



# 15 Spring viraemia of carp virus

## 15.1 Background

Spring viraemia of carp virus (SVCV) is the aetiological agent of spring viraemia of carp (also called infectious dropsy of carp or swim-bladder inflammation), an acute haemorrhagic viraemia in carp species and some other cyprinid and ictalurid species (Ahne et al. 2002; OIE 2021c). SVCV is also known as *Sprivirus cyprinus* and *Rhabdovirus carpio* (Fijan et al. 1971) and is a member of the genus *Sprivirus* in the family *Rhabdoviridae* (ICTV 2022).

SVCV was first reported in the former Yugoslavia in the 1970s (Fijan et al. 1971) and has since been recorded from all countries in Europe that experience low water temperatures during winter (OIE 2021e). The disease has also been reported in Asia, Africa and the Americas (Alexandrino, Tavares Ranzani-Paiva & Romano 1998; Asl et al. 2008; Dikkeboom et al. 2004; Garver et al. 2007; Goodwin 2002; OIE 2021e; Ortega et al. 2019; Soliman et al. 2008).

Infection with SVCV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (OIE 2021c) and is on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). Australia has a long history of passive surveillance and a strong system in place to detect incursions. SVCV is considered exotic to Australia.

## 15.2 Technical information

### 15.2.1 Agent properties

SVCV is a bullet-shaped, non-segmented, negative-sense, single-stranded RNA virus that measures 120–180 nm in length and 60–90 nm in diameter (Ahne et al. 2002). It is officially classified by the International Committee on Taxonomy of Viruses in the genus *Sprivirus*, subfamily *Alpharhabdovirinae* and family *Rhabdoviridae* (ICTV 2022).

The virion contains 5 structural proteins that are the RNA-dependent RNA polymerase (L protein), glycoprotein (G protein), nucleoprotein (N protein), phosphoprotein (P protein) and membrane protein (M protein) (Ahne et al. 2002). SVCV isolates can be divided into 4 genotypes (Ia, Ib, Ic, and Id) based on phylogenetic analyses of P and G gene sequences and these genotypes correlate with the geographical origin of isolates (Stone et al. 2003). As in other RNA viruses that can evolve rapidly, a high level of plasticity has been reported in the SVCV genome (Ashraf et al. 2016).

Disease outbreaks in carp generally occur between 11–17°C and rarely occur below 10°C or when the temperature exceeds 22°C (OIE 2021e). However, fish fry can be affected at temperatures as high as 23°C (Ahne et al. 2002). Intraperitoneal injection of *Cyprinus carpio* (common carp) with SVCV produced 100% mortality when fish were kept at 15–25°C water temperature but no mortality when kept at 30°C. In the same study, infected *Carassius auratus* (goldfish) and *Notemigonus crysoleucas* (golden shiner) showed mortality of 50–66% at 15°C and 20°C but no mortality at 25°C (Goodwin 2002).

The virus has been shown to remain viable outside the host for 5 weeks in river water at 10°C, for more than 6 weeks in pond mud at 4°C, reducing to 4 days in pond mud at 10°C ((Ahne 1976) cited in (OIE 2021e)). SVCV can retain infectivity for several months when frozen in medium containing 2–5%

serum. It is most stable at lower temperatures, with little loss of titre when stored for 1 month at –20°C or for 6 months at –30°C or –74°C ((Ahne 1976; De & Le Berr 1974) cited in (OIE 2021e)). SVCV can survive freeze-thaw cycles although infectivity is reduced (Ahne et al. 2002). Replication of SVCV occurs in the cytoplasm of cultured cells (fish, bird and mammalian origin) at temperatures of 4–31°C, with an optimum of about 20°C (Ahne et al. 2002).

SVCV is inactivated within 10 minutes by formalin (3%), chlorine (500 ppm), iodine (0.01%), sodium hydroxide (2%), UV (254 nm) and gamma irradiation (103 krad) (Ahne et al. 2002; Skall & Olesen 2011). It was also reported to be inactivated by heat exposure at 50°C for 1 hour and a three-log reduction in the virus load in cell culture was obtained by exposure to 45°C for 1 hour (Skall & Olesen 2011).

## 15.2.2 Epidemiology

### Host range

Species which fulfil the criteria for listing as a species susceptible to infection (N= natural exposure; E= experimental exposure) with SVCV in accordance with chapter 1.5 of the WOA *Aquatic animal health code* (WOAH Code) (OIE 2021a) include:

- *Abramis brama*<sup>N</sup> (bream) (Basic et al. 2009)
- *Aristichthys nobilis*<sup>N</sup> (bighead carp) (Shchelkunov & Shchelkunova 1989)
- *Carassius auratus*<sup>N,E</sup> (goldfish) (Alexandrino, Tavares Ranzani-Paiva & Romano 1998; Basic et al. 2009; Goodwin 2002; Jorgensen et al. 1989)
- *Ctenopharyngodon idella*<sup>N,E</sup> (grass carp) (Haenen & Davidse 1993; Shchelkunov & Shchelkunova 1989)
- *Cyprinus carpio*<sup>N,E</sup> (common carp, all varieties and subspecies) (Basic et al. 2009; Haenen & Davidse 1993)
- *Danio rerio*<sup>E</sup> (zebrafish) (Sanders, Batts & Winton 2003)
- *Notemigonus crysoleucas*<sup>E</sup> (golden shiner) (Goodwin 2002)
- *Pimephales promelas*<sup>E</sup> (fathead minnow) (Emmenegger et al. 2016; Misk et al. 2016)
- *Percocypris pingi*<sup>N</sup> (Jinsha barbel carp) (Zheng et al. 2018)
- *Rutilus kutum*<sup>E</sup> (Caspian white fish) (Ghasemi et al. 2014)
- *Rutilus rutilus*<sup>E</sup> (roach) (Haenen & Davidse 1993)
- *Silurus glanis*<sup>N</sup> (Wels catfish) (Fijan et al. 1984; Jorgensen et al. 1989).

Species for which there is incomplete evidence for susceptibility to infection (N= natural exposure; E= experimental exposure) according to WOAH include:

- *Acipenser baerii*<sup>N</sup> (Siberian sturgeon) (Vicenova et al. 2011)
- *Carassius carassius*<sup>N</sup> (Crucian carp) ((Kolbl 1975) cited in (Haenen & Davidse 1993))
- *Cynops orientalis*<sup>N</sup> (Chinese firebelly newt) (Hon, Lorch & Blehert 2016)
- *Esox lucius*<sup>N</sup> (pike) (Koutná et al. 2003; Vicenova et al. 2011)

- *Hypophthalmichthys molitrix*<sup>N</sup> (silver carp) (Gado, Saad & Omar 2015; Shchelkunov & Shchelkunova 1989)
- *Perca flavescens*<sup>E</sup> (yellow perch) (Emmenegger et al. 2016).

SVCV-positive PCR results or viral isolation in cell culture have also been reported in several species, however, no active infection was demonstrated (N= natural exposure; E= experimental exposure), including:

- *Ardea cinerea*<sup>E</sup> (heron) (Peters & Neukirch 1986)
- *Argulus foliaceus*<sup>E</sup> (fish lice) (Ahne 1985)
- *Catla catla*<sup>N</sup> (catla) (Asl et al. 2008)
- *Catostomus commersonii*<sup>E</sup> (white sucker) (Misk et al. 2016)
- *Cirrhinus merigala*<sup>N</sup> (Merigal carp) (Asl et al. 2008)
- *Labeo rohita*<sup>N</sup> (Rohu) (Asl et al. 2008)
- *Lepomis macrochirus*<sup>N</sup> (bluegill) (Phelps et al. 2012)
- *Leuciscus idus*<sup>N</sup> (orfe, ide) (Dixon, Hattenberger-Baudouy & Way 1994)
- *Micropterus salmoides*<sup>N</sup> (largemouth bass) (Phelps et al. 2012)
- *Notropis atherinoides*<sup>E</sup> (Emerald shiner) (Misk et al. 2016)
- *Oncorhynchus mykiss*<sup>E</sup> (Rainbow trout) (Emmenegger et al. 2016; Haenen & Davidse 1993)
- *Oncorhynchus nerka*<sup>E</sup> (Sockeye salmon) (Emmenegger et al. 2016)
- *Oncorhynchus tshawytscha*<sup>E</sup> (Chinook salmon) (Emmenegger et al. 2016)
- *Oreochromis niloticus*<sup>N, E</sup> (Nile tilapia) (Gado, Saad & Omar 2015; Soliman et al. 2008)
- *Penaeus vannamei*<sup>N</sup> (Pacific white shrimp) (Johnson et al. 1999; Lu, Loh & Nadala 1994)
- *Piscicola geometra*<sup>E</sup> (leech) (Ahne 1985)
- *Poecilia reticulata*<sup>E</sup> (guppy) (Bachmann & Ahne 1974)
- *Tinca tinca*<sup>N</sup> (tench) (Dixon, Hattenberger-Baudouy & Way 1994).

Common carp and ornamental varieties are the principal hosts for SVCV (Ahne, Kurath & Winton 1998; Fijan et al. 1971). There has been one confirmed report (PCR and sequencing) of SVCV in *A. baerii* (Siberian sturgeon) displaying clinical signs and mortality (Vicenova et al. 2011). The sturgeon isolate was genetically identical to the isolate recovered from clinically healthy *Cyprinus carpio koi* (koi carp) collected from the same aquaculture site (Vicenova et al. 2011).

Generally, young fish up to 1 year old are most susceptible to clinical disease, but all age groups can be affected (Emmenegger et al. 2016; OIE 2021e). SVCV DNA was detected in *A. baerii* fry (Vicenova et al. 2011).

SVCV has also been isolated from numerous non-fish species such as amphibians (*Cynops orientalis*) (Hon, Lorch & Blehert 2016), penaeid prawns (*Penaeus stylirostris* and *P. vannamei*) (Lu et

al. 1991), fish lice (*Argulus foliaceus*), leeches (*Piscicola geometra*) and herons (*Ardea cinerea*) (Pfeil-Putzien 1977) cited in (Ahne et al. 2002))(Ahne 1985; Peters & Neukirch 1986).

### Geographical distribution

SVCV was first isolated in former Yugoslavia and it has since been reported in most European countries and the United Kingdom (UK) ((Fijan et al. 1971) cited in (Kibenge & Godoy 2016)). It has also been reported in countries from Asia, Africa and the Americas including Brazil (Alexandrino, Tavares Ranzani-Paiva & Romano 1998), Canada (Garver et al. 2007), China (Liu et al. 2004), Egypt (Gado, Saad & Omar 2015; Soliman et al. 2008), India (Asl et al. 2008), Iran (Asl et al. 2008), Mexico (Ortega et al. 2019) and the United States of America (USA) (Dikkeboom et al. 2004; Goodwin 2002; Phelps et al. 2012).

### Prevalence

#### Sturgeon

During 1995–2008, fish showing clinical signs of a viral disease were collected from farms in the Czech Republic and SVCV was detected in 12% (n=178) of samples, including one from *A. baerii* (Vicenova et al. 2011).

#### Other fish

There are limited publications about SVCV prevalence in wild or farmed populations of fish. A survey carried out in Serbia over a 10-year period (1992–2002) detected SVCV in 31% (n=38) of carp hatcheries screened. In the survey, SVCV appeared for the first time during the spring of 1992 at water temperatures of 13–15°C, and then it was detected sporadically from year to year at different sites (Jeremić, Jakić-Dimić & Radosavljevic 2004). In wild *C. carpio* sampled from Hamilton Harbour, Lake Ontario in Canada in 2006, SVCV was detected at 60% prevalence (n=30) in tissue pools (kidney, spleen and encephalon from 5 fish/pool) (Garver et al. 2007).

Between 1977–2010, SVCV was confirmed on 108 occasions in England and Wales, with 65 of the cases occurring in sport fisheries and the majority of the remainder occurring in the ornamental fish sector (Taylor et al. 2013). In Egypt, SVCV prevalence was 43% (n=30) in *O. niloticus* from farms in 3 governorates (Soliman et al. 2008).

### Mortalities

#### Sturgeon

SVCV was reported to cause mortality in farmed *A. baerii* from South Bohemia, Czech Republic in 2006 but no numbers were given (Vicenova et al. 2011). No reports were found on mortalities in wild sturgeon due to SVCV.

#### Other fish

SVCV can cause mortalities of about 70% during springtime outbreaks in young carp but outbreaks do depend on the temperature of the water, age and condition of fish, population density, and stress factors (Ahne et al. 2002). High mortality typically occurs at water temperatures of 10–17°C (Ahne et al. 2002). Also, secondary and concomitant bacterial and/or parasitic infections can affect the mortality rate (Ahne et al. 2002).

Mortalities of 70% due to SVCV have been reported in wild *C. auratus* from Lago Nabuco, Brazil (Alexandrino, Tavares Ranzani-Paiva & Romano 1998). SVCV outbreaks in *C. carpio koi* farms in North Carolina and Virginia, USA in 2002 resulted in 15,000 fish deaths and a further 135,000 fish were

euthanised (Kurath & Emmenegger 2018). In spring 2002, mortalities over a 6-week period of an estimated 1,500 *C. carpio* were reported in Cedar Lake, Wisconsin, USA (Dikkeboom et al. 2004). In 2011, 200–300 dead or moribund *C. carpio* were observed in Minnehaha Creek, USA due to SVCV infection (Phelps et al. 2012). Mortalities of about 5% were reported in farmed *C. carpio*, *H. molitrix* and *O. niloticus* during an SVCV outbreak in Egypt (Gado, Saad & Omar 2015). In Sichuan Province, China, 35% mortality occurred in farmed *P. pingi* in 2016 due to SVCV (Zheng et al. 2018). In 2021, a SVCV outbreak in farmed *C. carpio* in Romania caused 30 fish deaths (OIE 2021g).

### Transmission

Transmission of SVCV is horizontal, either direct or vectorial (OIE 2021e). SVCV is shed in the mucus, faeces and urine of clinically infected fish and carriers. Waterborne transmission is believed to be the primary route of infection (Ahne et al. 2002; Ghasemi et al. 2014). In experimental waterborne infections, the incubation period was approximately 7 days and SVCV was shed in the mucus and faeces from 11 days post infection (dpi) (Ahne et al. 2002). Transmission from broodstock to progeny and egg associated transmission cannot be ruled out following isolation of SVCV from *C. carpio* ovarian fluid (Békési & Csontos 1985).

Surviving fish can become carriers of the virus for extended periods (OIE 2021e). For example, SVCV persisted in experimentally infected (by immersion) *C. carpio* for more than 10 weeks and those fish became carriers (Ahne et al. 2002). Factors affecting persistence and duration of the carrier state have not yet been studied (OIE 2021e).

Bloodsucking parasites like leeches, carp louse and fish eating-birds may serve as mechanical vectors. For example, the fish lice *Argulus foliaceus* and leech *Piscicola geometra* have been experimentally shown to transmit SVCV (Ahne 1985)((Pfeil-Putzien 1977) cited in (Ahne et al. 2002)). SVCV has also been isolated from samples of food which were regurgitated by *Ardea cinerea* (herons) at different times (30, 60 and up to 120 minutes) after feeding on infected fish (Peters & Neukirch 1986).

The introduction of SVCV into new areas has been primarily attributed to the movement of live animals (Ariel 2005; Taylor et al. 2013). Since 1996, SVCV has been imported into the UK on several occasions with shipments of cold-water ornamental fish from Asia, with consignments carrying the virus being intercepted in the airport and discovered during routine screenings (Ariel 2005; Taylor et al. 2013). In Denmark, SVCV was first isolated in 2002 from a batch of *C. carpio koi* from a pet shop that had been bought from a German wholesale distributor with many sources (Ariel 2005).

### Infectious dose

The minimum infectious dose of SVCV required to cause disease in susceptible species by experimental challenge or natural infection is not known. However, immersion of fry of *C. carpio*, *R. rutilus* and *C. idella* in a suspension containing SVCV at a final concentration of  $10^{5.8}$  TCID<sub>50</sub>/mL (water temperature 4–20°C) resulted in mortalities of 97%, 38% and 15%, respectively, by 35 dpi (Haenen & Davidse 1993). Bath exposure of fish to SVCV at a concentration of  $1 \times 10^5$  PFU/mL for 1–2 hours resulted in mean cumulative mortalities of 70–75% in *C. carpio koi* (weight 0.94–3.5 g), 5% in *O. mykiss* (weight 0.10 g) and 2–7% in *P. flavescens* (weight 0.6 g) (Emmenegger et al. 2016). Intraperitoneal injection of *N. atherinoides* (mean weight 3.5 g), *P. promelas* (mean weight 3 g) and *C. carpio koi* (mean weight 16 g) with 0.1 mL SVCV homogenate containing  $1 \times 10^6$  PFU resulted in cumulative mortalities of 43%, 53% and 33%, respectively (Misk et al. 2016). *R. frisii kutum* fingerlings bath exposed for 4 hours to SVCV at a concentration of  $6.5 \times 10^4$  TCID<sub>50</sub>/mL resulted in 85% mortality

(Ghasemi et al. 2014). In the same study, fingerlings challenged with SVCV orally (food soaked in solution containing  $6.5 \times 10^4$  TCID<sub>50</sub>/mL SVCV), by intraperitoneal injection (0.1ml inoculums at  $6.5 \times 10^4$  TCID<sub>50</sub>/mL viral concentration) and cohabitation induced cumulative mortalities of 10%, 75% and 55%, respectively (Ghasemi et al. 2014).

### 15.2.3 Pathogenesis

#### Tissue tropism

The virus appears to enter the host via the gills (Ahne 1978). A viraemia follows with the virus rapidly spreading through the bloodstream to the liver, kidney, spleen, brain and alimentary tract (Ahne & Wolf 1977; OIE 2021e).

#### Tissue titre

In natural infections, SVCV has been detected in the liver at  $10^{6.5}$  TCID<sub>50</sub>/g, kidney at  $10^{5.8}$  TCID<sub>50</sub>/g, brain at  $10^{4.3}$  TCID<sub>50</sub>/g, spleen at  $10^{3.8}$  TCID<sub>50</sub>/g and gills at  $10^{3.5}$  TCID<sub>50</sub>/g ((Fijan et al. 1971) cited in (Ahne & Wolf 1977)). In experimental infections, SVCV was reported in the liver at  $10^{6.8}$  TCID<sub>50</sub>/g, kidney at  $10^{5.2}$  TCID<sub>50</sub>/g, brain at  $10^{4.5}$  TCID<sub>50</sub>/g and spleen at  $10^{5.5}$  TCID<sub>50</sub>/g (Ahne & Wolf 1977). SVCV titres of  $10^{5.1}$  TCID<sub>50</sub>/mL and  $10^{6.4}$  TCID<sub>50</sub>/mL were found in experimentally infected *R. rutilus* and *C. carpio* fry, respectively (Haenen & Davidse 1993).

Experimental infection of *C. carpio koi* with  $1 \times 10^6$  PFU SVCV resulted in a viral copy number of  $2.6 \times 10^5$  at 3 dpi and  $2.6 \times 10^6$  at 34 dpi (Misk et al. 2016).

### 15.2.4 Diagnosis

#### Clinical signs

Infected fish typically concentrate around the water inlet or sides of a pond and their swimming speed, respiration rate and reactions to sensory stimulation are slowed down (Ahne et al. 2002). Typical clinical signs include darkened skin, exophthalmia, pale gills, abdominal distension (dropsy) and haemorrhages on the skin, base of the fins and the vent (Ahne et al. 2002; Haenen & Davidse 1993; OIE 2021e). The vent may also be swollen with trailing mucoid faecal casts (Ahne et al. 2002; Haenen & Davidse 1993; OIE 2021e). Fish can also be subclinically infected with SVCV (Ahne et al. 2002; Garver et al. 2007).

#### Pathology

SVCV can cause peritonitis, hyperaemia, ascites and a catarrhal or haemorrhagic enteritis. Viscera are oedematous and petechial haemorrhages occur in the heart, liver, kidneys, intestine, swim bladder, epithelium and skeletal muscle. Enlarged kidney and spleen have been observed. Multifocal hepatic necrosis with lymphocytes and histiocyte infiltration can be present in the liver. Diseased fish can also show inflammation and multifocal necrobiosis of the pancreas, spleen hyperplasia and degeneration of kidney and heart (Ahne 1978; Ahne et al. 2002).

SVCV has been detected in *A. baerii* which exhibited internal haemorrhages, a greyish-yellow liver showing tiny red spots and an enlarged, bright red spleen (Vicenova et al. 2011).

#### Testing

Chapter 2.3.9 of the WOA *Manual of diagnostic tests for aquatic animals* (WOAH Manual) provides details of the methods currently available for targeted surveillance and diagnosis of SVCV (OIE 2021e).

Cell culture (Fijan et al. 1983), RT-PCR (Stone et al. 2003), immunohistochemistry (Faisal & Ahne 1983), antigen enzyme-linked immunosorbent assay (Ag-ELISA) (Way 1991) and indirect fluorescent antibody test (IFAT) (Dixon & Longshaw 2005) are recommended for a presumptive diagnosis of clinically affected animals, despite the lack of validation data (OIE 2021e). Cell culture and RT-PCR followed by product sequencing are the recommended methods for confirmatory diagnosis of SVCV (OIE 2021e).

### **15.2.5 Treatment**

There is currently no safe and effective treatment available for SVCV (OIE 2021e).

### **15.2.6 Control and prevention**

Methods to control spring viremia of carp mainly rely on avoiding exposure to the virus coupled with good hygiene practices, including disinfection of eggs by iodophor treatment, disinfection of equipment and quarantining new fish brought into the facility (LaPatra et al. 2016; OIE 2021e). Reducing fish stocking density during winter and early spring is suggested to reduce the spread of the virus (OIE 2021e). A strain of *C. carpio* relatively resistant to SVCV was produced after several generational rounds but has not been repeated (Kirpichnikov et al. 1993). Following a control and eradication programme for SVCV initiated in 2005, the UK was recognised as free of the virus in 2010 (Taylor et al. 2013). The programme involved restrictions on imports of susceptible species from SVCV-positive countries, an increased focus on preventing illegal imports of large carp from mainland Europe (for the purpose of stocking into fisheries), a targeted surveillance programme for SVCV on fish farms and in imported fish, investigation of sites with reported disease outbreaks or suspicion of SVCV, culling stock at infected retail establishments and increasing awareness of the threat posed by imported fish to fisheries (Taylor et al. 2013).

### **15.2.7 Impact of the disease**

SVCV is recognised as having a serious economic impact due to its association with major disease epizootics in cultured carp populations (OIE 2021e). Spring viremia of carp was reported as one of the main disease problems in cyprinids in Europe in 2020 (EURL for Fish and Crustacean Diseases 2021). Carp is one of the most commercially important species in Europe. The Federation of European Aquaculture producers reported approximately 62,000 tonnes in carp production in 2019 (EURL for Fish and Crustacean Diseases 2021). In European aquaculture, mortality rates of young carp due to SVCV can reach up to 70% during springtime outbreaks, but the yearly losses of older fish are usually below 30% (Ahne et al. 2002). The main impact of SVCV to farms, dealers and retailers in the UK was attributed to the necessity to cull all stock and disinfect premises in order to continue trading (Taylor et al. 2013). In fisheries, impact was attributed to mortality and a subsequent loss in angling revenue (Taylor et al. 2013). The financial loss of UK cases of SVCV varied between sectors, from an estimated £20–30,000 for retail outlets, £30,000+ to a fishery and £20–230,000 to farms ((McGregor 1997) cited in (Taylor et al. 2013)). More recent reports on the economic impacts of SVCV are not available.

### **15.2.8 Current biosecurity measures**

There are no biosecurity measures for live sturgeon and their reproductive material as import is not permitted.

There are biosecurity measures to manage the risk of SVCV in imported ornamental fish for display purposes (see [Appendix D](#)).

## 15.3 Risk assessment

Based on [chapter 4](#) and the technical information about SVCV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of SVCV meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 15.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that SVCV is present in all source countries.
- It is expected SVCV can infect sturgeon life stages that would be exported to Australia.
- There has only been one report of SVCV in *A. baerii* and the clinically affected sturgeon were farmed at the same aquaculture site as infected, clinically healthy koi carp (Vicnova et al. 2011).
- The prevalence of SVCV in farmed or wild sturgeon is unknown.
- The prevalence of SVCV can be up to 30% in other farmed fish populations and 60% in wild fish.
- There are no reports of SVCV associating with sturgeon reproductive material but SVCV has been isolated from *C. carpio* ovarian fluid (Békési & Csontos 1985).
- The viral load of SVCV in infected imported live sturgeon or their reproductive material is likely to be sufficient to cause infection in susceptible species.
- SVCV can remain viable in the environment for several weeks.
- Inspection may detect sturgeon showing clinical signs that are typical of infection with SVCV and remove them before export. Sturgeon subclinically infected or carrier fish would not be identified through visual inspection.
- Sturgeon reproductive material infected or contaminated with SVCV are unlikely to be detected during inspection because there would be no clinical signs.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of SVCV was estimated to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

### 15.3.2 Exposure assessment

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

- SVCV can be transmitted horizontally via water.



- SVCV can survive in the environment for up to 5–6 weeks.
- SVCV would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- SVCV can infect varieties and subspecies of carp and *R. rutilus* present in Australia.
- Aquaculture species most likely to be polycultured with imported sturgeon such as trout and other salmonids do not appear to be susceptible to SVCV. However, they may act as vectors for SVCV.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the range of 11–22°C when SVCV outbreaks can occur (OIE 2021e).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infected sturgeon will be certain to be exposed to viable SVCV.
- Introduction of SVCV into the wild may occur by direct release of imported live sturgeon or its associated wastes from the aquaculture facility into natural waters. This would be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for fish escapes or waste management.
- Wild susceptible species would be less abundant than susceptible species in aquaculture facilities. Despite this, wild susceptible species would be expected to be exposed to SVCV released into natural waters due to hosts being present in Australian waters.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to SVCV in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

The partial likelihood of exposure to each exposure group to SVCV in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

### 15.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to SVCV in **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

The partial annual likelihood of entry and exposure of each exposure group to SVCV in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

### 15.3.4 Consequence assessment

#### Partial likelihood of establishment and spread (PLES)

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

- SVCV can be transmitted horizontally via water. SVCV may be transmitted from broodstock to progeny.
- SVCV can remain viable outside the host for 5–6 weeks under certain conditions.
- SVCV can infect fish species present in Australia, including common carp and ornamental varieties and *R. rutilus*.
- Young fish up to 1 year old are most susceptible to clinical disease but all age groups can be affected.
- Aquaculture species most likely to be polycultured with imported sturgeon such as trout do not appear to be susceptible to SVCV but may act as vectors for SVCV.
- Other species that may act as SVCV vectors present in Australia include *P. reticulata* and *T. tinca*.
- There is evidence that survivors of SVCV are persistently infected with virus and may retain the virus for long periods without showing clinical signs of disease.
- Outbreaks in carp are typically observed between 11–17°C but infections could be subclinical when water is at low temperatures (<10°C).
- SVCV establishment, following a given quantity of SVCV entering the environment of an exposure group, is likely for farmed susceptible species. This is due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- Live sturgeon or their reproductive material could be moved to other aquaculture facilities in Australia. Species polycultured with SVCV-infected sturgeon or in the same water, could also be moved to other facilities. It is expected that SVCV would establish in these facilities if present in the animals or reproductive material being translocated.
- Each state and territory have translocation protocols for aquaculture animals, which includes consideration of SVCV.
- If SVCV were to establish on a farm it could spread to neighbouring farms and wild populations through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of SVCV be suspected, and response measures initiated immediately. However, SVCV is effectively transmitted through water, and farms which share a common water source with an infected population may be exposed.
- The likelihood of SVCV spread from farms to neighbouring farms or wild populations via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals,

however SVCV could spread this way. SVCV could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.

- Australian native species of finfish are not considered susceptible to SVCV but there is a significant wild population of feral cyprinids in Australia that may succumb to infection.
- If one or more index cases of SVCV were to occur in the wild, establishment and spread would be more likely than on a farm because the large population of carp in Australia increases the opportunities for transmission. The ability of fish to be subclinically infected with SVCV and to remain carriers after surviving an infection also aids its spread.
- The likelihood of SVCV in a wild population spreading to its natural geographic limits is greater compared to other hazards with moderate host ranges, for example, infectious haematopoietic necrosis virus IHNV. However, once established, SVCV can spread rapidly over broad regions. For example, once established in the UK, SVCV spread throughout the country (Taylor et al. 2013).
- If SVCV were to establish in the wild, especially in waters around aquaculture facilities, it may easily spread to farms through water intake due to SVCV being able to survive in the environment and being transmissible through water.

### Conclusion

Based on this information and using the descriptors in Table 4, the partial likelihood of establishment and spread of SVCV in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**Moderate.**
- Wild susceptible species—**High.**

### Determining adverse impacts resulting from the outbreak scenario

The factors considered relevant when determining the adverse impacts resulting from establishment and spread of SVCV were that:

#### *Direct effects*

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

- Spring viremia of carp is primarily a disease of cyprinids, including varieties and subspecies of carp. The domestic koi carp industry would be significantly affected by an outbreak of SVCV.
- There is one report of SVCV in sturgeon and it was associated with mortality. Production losses in sturgeon aquaculture due to SVCV would be significant considering sturgeon require >3 years to reach sexual maturity.
- SVCV is not expected to impact wild fisheries in Australia except for the commercial wild-caught carp industry.
- Based on the impact of SVCV in European aquaculture, the establishment and spread of SVCV in Australia would be expected to cause minor impacts at the state or territory 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*

- SVCV has been detected in wild fish species overseas and there have been reports of clinical disease and mortalities in the wild due to SVCV.
- Carp would likely be the only wild susceptible species present in Australia affected by an outbreak of SVCV and carp are considered one of the worst introduced pest species in the country (Australian Government & FRDC 2022). An outbreak of SVCV could have up to 70% mortality, which would result in a significant biomass that could affect the living environment if not effectively cleaned-up (Silva, Bell & Baumgartner 2019).
- The direct impact of SVCV 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*

- Infection with SVCV is listed as a notifiable disease by WOA and it is included on Australia's *National list of reportable diseases of aquatic animals*. States and territories would be required to report the occurrence of SVCV.
- If SVCV 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 SVCV from wild populations, as occurred in the UK, would be difficult and expensive.
- If SVCV was confirmed at a farm, then attempts at eradication would 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 restriction area were put in place for an outbreak of SVCV, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- Carp completely 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). The biomass of carp in south-eastern Australia was estimated to be 205,774 tonnes (Stuart et al. 2021). An outbreak of SVCV could have up to 70% mortality, which would result in a significant biomass that would be required to be effectively cleaned-up. The biomass clean-up may be even greater than that estimated for an CyHV-3 outbreak since SVCV can effectively spread via water (Silva, Bell & Baumgartner 2019). Therefore, the cost of clean-up would be significant.
- Eradication and control of SVCV 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*

- If movement restriction orders were put in place it would have indirect impacts on other industries such as commercial wild catch carp fisheries and the fishery for fertiliser and bait.
- SVCV-infected fish may show clinical signs which would affect their marketability.
- SVCV 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*

- SVCV is a WOAHL-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to SVCV to avoid the possible introduction of SVCV.
- Ornamental finfish for export may need to be tested and free of SVCV as part of export certification if SVCV was to become established in Australia.
- The impacts of SVCV 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*

- Spring viremia of carp is primarily a disease of cyprinid species and could cause significant mortality in wild carp.
- Significant mortalities would result in a significant biomass that if not effectively cleaned-up could impact local biodiversity.
- There are no species listed as endangered in Australia that are known to be susceptible to SVCV.
- The impact of SVCV establishment and spread on the biodiversity of the environment is expected to be minor at the state and territory 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 SVCV are recreationally fished in Australia and could be affected by mortalities due to infection or movement restriction areas put in place which may impact on social amenity.
- Large scale mortalities may have a temporal detrimental effect on social amenity if dead fish are not effectively removed and disposed of properly.
- In local areas where recreational fishing or ornamental fish trade are a major industry, a SVCV outbreak could cause loss of business and welfare concerns.
- The social impacts of SVCV establishment and spread are expected to be minor at the district or region level.

Table 23 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of SVCV. 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 23 Overall impact of establishment and spread of SVCV for the outbreak scenario**

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	State or territory	Minor	D
	The environment (native animals/plants, and non-living environment)	National	Minor	E

Effects	Criteria	Level	Impact	Score
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)	District or region	Minor	C
	Economic (international trade effects)	State or territory	Minor	D
	Environment (biodiversity, endangered species and the integrity of ecosystems)	State or territory	Minor	D
	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 SVCV was estimated to be **moderate**.

### 15.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for SVCV 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

### 15.3.6 Determination of the partial annual risk

The partial annual risk of SVCV entry, establishment and spread from **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

The partial annual risk of SVCV entry, establishment and spread from **imported sturgeon reproductive material** for each exposure group was similarly and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

### 15.3.7 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 risk associated with SVCV was found to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

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

## 15.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for SVCV in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 15.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 24 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of SVCV but which on their own do not achieve Australia's ALOP for SVCV in imported live sturgeon or their reproductive material.

**Table 24 Biosecurity measures that on their own do not achieve Australia's ALOP for SVCV**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Disease-free stock	<b>Yes:</b> Determination of SVCV freedom would need to be to a standard consistent with that recommended by the World Organisation for Animal Health (WOAH), or equivalent.
2	Post-arrival quarantine (PAQ)	<b>Yes:</b> The stress of transport can induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
3	Post-arrival batch testing	<b>Yes:</b> There are RT-PCR methods available to detect SVCV (OIE 2021e). Under this scenario, testing is conducted under departmental control and oversight.

### 15.4.2 Biosecurity measures that in combination achieve Australia's ALOP

A combination of biosecurity measures 1, 2 and 3 when applied to **imported live sturgeon** would reduce the likelihood of entry of SVCV from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1, 2 and 3 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of SVCV from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

# 16 Sturgeon alloherpesviruses

## 16.1 Background

Sturgeon alloherpesviruses can cause serious disease in farmed and wild sturgeon (Hanson et al. 2016; Hedrick et al. 1991a; Johnston et al. 2022; Walker et al. 2022; Watson et al. 1995). Sturgeon alloherpesviruses include:

- Acipenserid herpesvirus 1 (AciHV1) (Hedrick et al. 1991a)
- Acipenserid herpesvirus 2 (AciHV2) (Watson et al. 1995)
- Lake sturgeon herpesvirus 1 (LSHV-1) (Walker et al. 2022).
- Lake sturgeon herpesvirus 2 (LSHV-2) (Johnston et al. 2022).

Sturgeon alloherpesviruses are only known to infect sturgeon species and have been reported from North America and Europe (Hedrick et al. 1991b; Johnston et al. 2022; LaPatra et al. 2014; Shchelkunov et al. 2009; Walker et al. 2022; Watson et al. 1995).

Infection with sturgeon alloherpesviruses is not listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (OIE 2020) and is not on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). Sturgeon alloherpesviruses are considered exotic to Australia.

## 16.2 Technical information

### 16.2.1 Agent properties

Sturgeon alloherpesviruses are icosahedral, enveloped, double-stranded DNA viruses (Johnston et al. 2022; Walker et al. 2022; Waltzek et al. 2009). AciHV1 was the first herpesvirus to be identified in sturgeon and is 230 nm in diameter (Hedrick et al. 1991a). AciHV2 (176–250 nm) was isolated a few years later and is currently the only sturgeon alloherpesvirus officially classified by the International Committee on Taxonomy of Viruses into the genus *Ictalurivirus*, family *Alloherpesviridae* (ICTV 2020; Shchelkunov et al. 2009; Watson et al. 1995). AciHV1 and AciHV2 were previously referred to as white sturgeon herpesvirus types 1 and 2, respectively (Hedrick et al. 1991a; Kurobe et al. 2008; Watson et al. 1995). LSHV-1 and LSHV-2 have only recently been identified and are approximately 85 nm in diameter (Johnston et al. 2022; Walker et al. 2022).

Serologic and phylogenetic evidence suggests that AciHV1 and AciHV2 are only distantly related (Kelley et al. 2005; Kurobe et al. 2008; Waltzek et al. 2009; Watson et al. 1995). AciHV2 is most closely related to Ictalurid herpesvirus 1 and 2 of catfish (Doszpoly et al. 2008; Kelley et al. 2005). A virus showing high similarity to AciHV2, Siberian sturgeon herpesvirus (SbSHV), was isolated in the Russian Federation in 2006 and is assumed to be a strain of AciHV2 (Doszpoly et al. 2017; Doszpoly & Shchelkunov 2010; Shchelkunov et al. 2009). LSHV-1 and LSHV-2 are most closely related to AciHV1 (Johnston et al. 2022; Walker et al. 2022).

There is little information on the environmental conditions which induce outbreaks of sturgeon alloherpesviruses. Morbidity and mortality have been reported at water temperatures of 11–19°C (Hedrick et al. 1991a; Johnston et al. 2022; Shchelkunov et al. 2009; Watson et al. 1995). Under



laboratory conditions, viral replication of AciHV1 on white sturgeon skin cell cultures occurs at temperatures between 10–20°C with the optimum being 15°C (Hedrick et al. 1991a). AciHV2 induced a cytopathogenic effect on white sturgeon spleen cells over 5–7 days at 15°C or 2–3 days at 20°C (LaPatra et al. 2014). LSHV-2 could be cultured on white sturgeon × lake sturgeon cells at 15°C (Johnston et al. 2022).

No reports on the stability of AciHV1 or AciHV2 were found. However, Cyprinid herpesvirus 3 (CyHV-3), another member of the family *Alloherpesviridae*, remains viable in water for 4–21 hours (Perelberg et al. 2003) and is sensitive to ultraviolet radiation, temperature and common disinfectants (Kasai, Muto & Yoshimizu 2005; OIE 2021c). AciHV-1, AciHV-2 and LSHV-2 survive freezing as stocks stored at –70°C and –80°C could induce infections when used in challenge studies (Hedrick et al. 1991a; Johnston et al. 2022; Watson et al. 1995).

## 16.2.2 Epidemiology

### Host range

In general, alloherpesviruses display a high level of host specificity and appear to cause disease in only one species of fish or in closely related members of the same genus (Hanson, Dishon & Kotler 2011).

Species which are reported to be susceptible to infection (N= natural exposure; E= experimental exposure) with AciHV1 include:

- *Acipenser transmontanus*<sup>N, E</sup> (white sturgeon) (Hedrick et al. 1991a; Kurobe et al. 2008).

Infection with AciHV1 has been observed in fry, fingerlings and juveniles (Hanson, Dishon & Kotler 2011; Hedrick et al. 1991a).

Species which are reported to be susceptible to infection (N= natural exposure; E= experimental exposure) with AciHV2 include:

- *Acipenser baerii*<sup>N, E</sup> (Siberian sturgeon) (Shchelkunov et al. 2009)
- *Acipenser brevirostrum*<sup>N</sup> (shortnose sturgeon) (Kelley et al. 2005; LaPatra et al. 2014)
- *Acipenser ruthenus* × *Huso huso* hybrid<sup>N</sup> (Bester) (Shchelkunov et al. 2009)
- *Acipenser transmontanus*<sup>N, E</sup> (white sturgeon) (Kurobe et al. 2008; Watson et al. 1995)
- *Scaphirhynchus albus*<sup>E</sup> (pallid sturgeon) (Kurobe et al. 2008)
- *Scaphirhynchus platyrhynchus*<sup>E</sup> (shovelnose sturgeon) (Kurobe et al. 2008).

Infection with AciHV2 has been observed in fingerlings, juveniles and adults (Mugetti et al. 2020b; Shchelkunov et al. 2009).

Species which are reported to be susceptible to infection (N= natural exposure; E= experimental exposure) with LSHV-1 include:

- *Acipenser fulvescens*<sup>N</sup> (lake sturgeon) (Walker et al. 2022).

Species which are reported to be susceptible to infection (N= natural exposure; E= experimental exposure) with LSHV-2 include:

- *Acipenser fulvescens*<sup>N, E</sup> (lake sturgeon) (Johnston et al. 2022).

LSHV-1 and LSHV-2 were detected in adults and LSHV-2 could experimentally infect juveniles (Johnston et al. 2022; Walker et al. 2022).

### Geographical distribution

AciHV1 has only been reported from farmed *A. transmontanus* in California, United States of America (USA) and Italy (Hedrick et al. 1991b; Kelley et al. 2005; Kurobe et al. 2008).

AciHV2 has been recorded from wild and farmed sturgeon in Canada and the USA (Kelley et al. 2005; Kurobe et al. 2008; LaPatra et al. 2014). SbSHV that is assumed to be a strain of AciHV2 has been reported in the Russian Federation (Doszpoly et al. 2017; Doszpoly & Shchelkunov 2010).

LSHV-1 and LSHV-2 have only been detected in wild *A. fulvescens* in Wisconsin and Michigan, USA, respectively (Johnston et al. 2022; Walker et al. 2022).

### Prevalence

No reports of AciHV1 prevalence in wild or farmed sturgeon were found.

AciHV2 was detected in 93% (n=56) of *A. transmontanus* grow out tanks from 3 farms in California, USA during a study conducted between 1997–1998 (Georgiadis et al. 2000).

LSHV-1 was detected at a prevalence of 92% (n=42) in skin lesion samples collected from 49 wild *A. fulvescens* captured in 2017–2021 across 4 rivers in Wisconsin, USA (Walker et al. 2022). In 2019, LSHV-2 was reported in skin lesions sampled from wild adult *A. fulvescens* from the Lake Huron and Lake Erie watersheds of the Great Lakes basin at a prevalence of 50% (n=8) and 100% (n=1), respectively (Johnston et al. 2022). A follow-up survey of LSHV-2 in wild *A. fulvescens* from the Lake Huron watershed in 2020 reported the virus at a prevalence of 50% (n=12) (Johnston et al. 2022).

### Mortalities

AciHV1 and AciHV2 have been reported to cause up to 50% mortality in young sturgeon (less than 6 months of age) (Goodwin 2012). AciHV1 infection in farmed *A. transmontanus* in California, USA caused up to 50% mortalities (Hedrick et al. 1991a). In 2006, an AciHV2 outbreak occurred in a hatchery in Tver Province, the Russian Federation, that resulted in mass mortality (up to 100%) of fry and fingerlings among several sturgeon species, particularly in *A. baerii* (Doszpoly & Shchelkunov 2010). Experimental infections of sturgeon with AciHV2 induced mortalities of 40–100% (Shchelkunov et al. 2009; Watson et al. 1995).

No reports of LSHV mortalities were found.

### Transmission

The primary mode of transmission for sturgeon alloherpesviruses is likely horizontal via water, including from fish with subclinical infection (Hedrick et al. 1991a; Johnston et al. 2022; Watson et al. 1995). Although LSHV-1 was found in association with skin lesions, its pathogenic potential has yet to be determined (Walker et al. 2022). It is unknown if transmission from broodstock to progeny can occur. However, it has been speculated that this transmission does occur in fish herpesviruses since transmission via the gametes from adult carriers was reported for the herpesvirus found in channel catfish *Ictalurus punctatus* (Wise et al. 1988). Additionally, AciHV2 has been isolated from gonadal tissue of sexually mature *A. transmontanus* showing no clinical signs of disease (Watson et al. 1995).

AciHV2 infection may cause a latent carrier state in surviving fish (Watson et al. 1995). AciHV2 has been recovered from experimentally infected *A. baerii* fingerling survivors at 70 days post infection (dpi) and from moribund 2-year-old fish at 56 dpi (Shchelkunov et al. 2009). Herpesviruses tend to cause disease in young immunologically naïve animals and then persist in latency for long periods, probably the lifetime of their fish hosts (Hedrick & Sano 1989). Those carrier fish, when stressed, may release new virus into the water and infect fish not previously exposed. Those newly infected fish may develop a disease and spread the virus to other fish; they also become carriers and may infect other fish later. Carrier fish make the control of herpesviruses in fish very difficult (Goodwin 2012).

The spread of sturgeon alloherpesviruses into new areas is likely a result of the movement of live animals. For example, it has been suggested that AciHV2 spread to Italy via numerous shipments of live *A. transmontanus* fry from the USA, a trade that was very active in the early 1990s (Kurobe et al. 2008).

### Infectious dose

Experimental challenge (by immersion) of *A. transmontanus* (mean weight 12.8 g) with AciHV1 at a concentration of  $10^{5.3}$  TCID<sub>50</sub>/mL for 30 minutes resulted in 35% cumulative mortality by 30 dpi (Hedrick et al. 1991a).

An immersion challenge of *A. transmontanus* (weight 1.5 g) with AciHV2 at concentrations of  $10^5$  and  $10^4$  TCID<sub>50</sub>/mL for 1 hour resulted in a cumulative mortality of 80% within 42 dpi (Watson et al. 1995). However, a challenge with  $10^3$  TCID<sub>50</sub>/mL failed to induce any clinical signs or mortality in *A. transmontanus*, although chronic subclinical infections were detected in most survivors at 70 dpi (Watson et al. 1995). *A. baerii* fingerlings (mean weight 12.5 g) experienced 100% mortality by 14 dpi following bath exposure to AciHV2 at a concentration of  $10^{4.7}$  TCID<sub>50</sub>/mL for 1 hour (Shchelkunov et al. 2009). Bath exposure of 2 year old *A. baerii* (weight 300–400 g) in a suspension of AciHV2 at a concentration of  $10^{3.8}$  TCID<sub>50</sub>/mL for 1 hour resulted in 90% morbidity and 36.4% mortality by 35 dpi (Shchelkunov et al. 2009).

Juvenile *A. fulvescens* (mean weight 1.2 g) bath exposed to  $1.28 \times 10^6$  TCID<sub>50</sub>/mL LSHV-2 for 1 hour resulted in severe disease and 33% mortality by 112 dpi (Johnston et al. 2022).

## 16.2.3 Pathogenesis

### Tissue tropism

AciHV1 has been associated with infections of the integument and oropharyngeal mucosa (Hedrick et al. 1991a). The integument and the mucosal and respiratory epithelium of the oropharyngeal cavity are reported to be the primary target tissues of AciHV2 (Watson et al. 1995). AciHV2 has also been detected in the kidney, spleen, liver and heart of infected sturgeon (Watson et al. 1995). LSHV-2 is associated with infection of the skin (Johnston et al. 2022).

### Tissue titre

Following experimental infection of *A. transmontanus* (by immersion), the AciHV2 concentrations in dead sturgeon ranged from  $10^{6.4}$ – $10^{6.9}$  TCID<sub>50</sub>/g in siphon/operculum, fins and barbels,  $10^{4.5}$ – $10^{5.5}$  TCID<sub>50</sub>/g in heart, gill, spleen, kidney and oesophagus and  $<10^{4.0}$  TCID<sub>50</sub>/g in liver and brain tissues (Watson et al. 1995). The AciHV2 mean titre in experimentally infected *A. baerii* was highest ( $>10^{9.1}$  TCID<sub>50</sub>/g) in skin mucus, fin, gill, skin and mouth tissues followed by liver ( $10^{7.85}$  TCID<sub>50</sub>/g), kidney

( $10^{6.25}$  TCID<sub>50</sub>/g), spleen ( $10^{5.68}$  TCID<sub>50</sub>/g), barbel ( $10^{5.35}$  TCID<sub>50</sub>/g), brain ( $10^{4.85}$  TCID<sub>50</sub>/g) and hind gut contents ( $10^{4.27}$  TCID<sub>50</sub>/g) (Shchelkunov et al. 2009).

There were no reports of AciHV1 or LSHV tissue titres.

## 16.2.4 Diagnosis

### Clinical signs

Affected sturgeon display erratic swimming, lethargy, anorexia, raised spherical mucoid skin lesions on the head, body and fins and hyperaemia of the ventral scutes, mouth and anus (Johnston et al. 2022; Shchelkunov et al. 2009; Walker et al. 2022; Watson et al. 1995). Multiple haemorrhages on the ventral part of the rostrum, around the mouth and on the abdominal and lateral body surfaces have also been observed along with erosion of the caudal fin (Johnston et al. 2022; Shchelkunov et al. 2009). In some cases, infected sturgeon show no clinical signs (Hedrick et al. 1991a; Watson et al. 1995).

Concurrent infections of AciHV2 with white sturgeon iridovirus (WSIV) (Georgiadis et al. 2000) and *Streptococcus iniae* (Soto et al. 2017b) have been reported in cultured *A. transmontanus*.

### Pathology

Histological changes caused by sturgeon alloherpesviruses include epidermal cell necrosis (AciHV1 and LSHV-2) and epidermal or branchial hyperplasia (AciHV1, AciHV2, LSHV-1 and LSHV-2) (Hanson, Dishon & Kotler 2011; Johnston et al. 2022; Walker et al. 2022). The nuclei are enlarged, lobulated and hypochromatic with marginated chromatin while the cytoplasm appears depleted and vacuolated (Hedrick et al. 1991a; Johnston et al. 2022; LaPatra et al. 2014; Watson et al. 1995). AciHV1-infected sturgeon can also present with fluid-filled stomachs and intestines (Hedrick et al. 1991a). Pale internal organs, an inflamed hind gut and enlarged gall bladder have been observed with some AciHV2 infections (Shchelkunov et al. 2009).

### Testing

Isolation of sturgeon alloherpesviruses by cell culture and observation of cytopathic effects has been used for diagnosis (Hedrick et al. 1991a; Johnston et al. 2022; Shchelkunov et al. 2009; Watson et al. 1995). PCR and sequencing have also been used to confirm the presence of sturgeon alloherpesviruses (Dospoly et al. 2008; Dospoly & Shchelkunov 2010; Johnston et al. 2022; Kelley et al. 2005; Kurobe et al. 2008; Walker et al. 2022; Waltzek et al. 2009).

## 16.2.5 Treatment

There are no treatments available for sturgeon alloherpesviruses.

## 16.2.6 Control and prevention

Selection of specific broodstock, stocking with sturgeon that survived outbreaks of viral disease, using all-in, all-out production, and decreasing stocking densities have been reported as factors that may reduce morbidity and mortality after exposure to AciHV2 (Georgiadis et al. 2000). The same management practices would be expected to minimise infection with AciHV1, LSHV-1 and LSHV-2.

## 16.2.7 Impact of the disease

AciHV1 can cause serious losses of hatchery fry, fingerlings and juvenile sturgeon (Hanson, Dishon & Kotler 2011; Hedrick et al. 1991a). AciHV2 has been associated with morbidity and mortality in farmed juveniles and subadult sturgeon (Watson et al. 1995). AciHV2, together with WSIV, are

considered to be the 2 most important viruses limiting survivability of juvenile *A. transmontanus* (Georgiadis et al. 2000; LaPatra et al. 2014). There are no reports on economic losses due to sturgeon alloherpesviruses. However, any economic losses would include the commercial value of the affected sturgeon as both potential broodstock and caviar producers.

### 16.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon and their reproductive material as import is not permitted.

## 16.3 Risk assessment

Based on [chapter 4](#) and the technical information about sturgeon alloherpesviruses presented in this chapter, a risk assessment was completed for AciHV1 and AciHV2 only.

Infection with LHSV-1 and LHSV-2 is an emerging disease and as such, evidence about the susceptibility of sturgeon is limited. LSHV-1 has yet to be shown as the causative disease agent of the clinical symptoms seen in infected sturgeon and with only 2 publications on LSHV, much is still unknown about the prevalence, mortality rates, infectious dose, tissue titre and diagnostics of the viruses. The department considers there is insufficient information regarding LSHV-1 and LSHV-2 to conduct a risk assessment and will continue to monitor the situation with respect to these hazards. The department routinely analyses ongoing media and scientific literature about biosecurity issues for all animal species to monitor biosecurity risks. The scientific information is regularly assessed by technical experts and if new information about a biosecurity risk is identified, the department reviews the risk further and acts when necessary. Should new information become available about LSHV-1 and LSHV-2, the department will consider the information and if appropriate, this risk assessment will be expanded to include LSHV-1 and LSHV-2.

A summary of the risk assessment values for determining if the overall annual risk of AciHV1 and AciHV2 meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 16.3.1 Entry assessment

The key points considered relevant when conducting the entry assessment for AciHV1 and AciHV2 were that:

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that AciHV1 and AciHV2 are present in all source countries.
- AciHV1 and AciHV2 infect sturgeon that would be of a life stage exported to Australia.
- There is only one report of AciHV2 prevalence in farmed *A. transmontanus* from California, USA, of 93% (Georgiadis et al. 2000). There are no reports of AciHV1 prevalence in farmed sturgeon.
- The prevalence of AciHV1 and AciHV2 in wild sturgeon is unknown.
- AciHV2 has been associated with sturgeon gonadal tissues (Watson et al. 1995). In the absence of evidence it will be assumed that sturgeon reproductive material can be infected since transmission via the gametes from adult carriers was reported for the herpesvirus found in channel catfish *Ictalurus punctatus* (Wise et al. 1988).

- It is unknown if AciHV1 or AciHV2 can remain viable outside the host. However, other members of the family *Alloherpesviridae* such as CyHV-3 can only survive in water for up to one day.
- The viral load of AciHV1 and AciHV2 in infected imported live sturgeon or their reproductive material is likely to be sufficient to cause infection in susceptible species.
- Inspection may detect sturgeon showing clinical signs that are typical of AciHV1 and AciHV2 and remove them before export. Sturgeon subclinically infected and carrier fish would not be identified by visual inspection.
- Sturgeon reproductive material infected or contaminated with AciHV1 and AciHV2 are unlikely to be detected during inspection because there would be no clinical signs.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of AciHV1 and AciHV2 was estimated to be:

- Imported live sturgeon—**High**.
- Imported sturgeon reproductive material—**High**.

### 16.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for AciHV1 and AciHV2 were:

- AciHV1 and AciHV2 can be transmitted horizontally via water.
- AciHV1 and AciHV2 would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- AciHV1 and AciHV2 only infect sturgeon.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are not susceptible to AciHV1 and AciHV2.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the water temperature range of 11–19°C which AciHV1 and AciHV2 induce mortality (Shchelkunov et al. 2009).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infected sturgeon will be certain to be exposed to viable AciHV1 and AciHV2.
- Introduction into the wild may occur by direct release of imported live sturgeon. Release of contaminated associated wastes from the aquaculture facility into natural waters would also be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for waste management that would exclude AciHV1 and AciHV2 from discharges. However, there are no known wild sturgeon populations in Australian waters.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to AciHV1 and AciHV2 in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

The partial likelihood of exposure of each exposure group to AciHV1 and AciHV2 in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

### **16.3.3 Determination of the partial annual likelihood of entry and exposure**

The partial annual likelihood of entry and exposure of each exposure group to AciHV1 and AciHV2 in **imported live sturgeon** 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:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

The partial annual likelihood of entry and exposure of each exposure group to AciHV1 and AciHV2 in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

### **16.3.4 Consequence assessment**

#### **Partial likelihood of establishment and spread (PLES)**

The key points considered relevant when determining the partial likelihood of establishment and spread for AciHV1 and AciHV2 were:

- AciHV1 and AciHV2 can be transmitted horizontally via water. It is assumed transmission from broodstock to progeny can occur.
- It is unknown how long AciHV1 and AciHV2 can remain viable outside the host.
- It is expected that susceptible host animals in contact with AciHV1- and AciHV2-infected sturgeon would receive an infectious dose.
- AciHV1 and AciHV2 only infect sturgeon, which are not yet found in Australia.
- There is evidence that survivors of AciHV1 and AciHV2 are persistently infected and may retain the virus for periods without showing clinical signs of disease.
- Outbreaks of AciHV1 and AciHV2 typically occur at water temperatures of 11–19°C.
- The likelihood of AciHV1 and AciHV2 establishment, following a given quantity of virus entering the environment of an exposure group, is greatest for farmed susceptible species. This is due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- If infected live sturgeon or their reproductive material were moved to another aquaculture facility in Australia it is likely that AciHV1 and AciHV2 would establish in these facilities.

- Each state and territory have translocation protocols for aquaculture animals, but they do not include consideration of AciHV1 and AciHV2.
- If AciHV1 and AciHV2 were to establish on a farm it could spread to neighbouring farms through wastewater. This spread would be moderated by dilution effects, the virus survival time in the environment and implementation of biosecurity measures should an incursion of AciHV1 and AciHV2 be suspected and response measures initiated immediately. However, AciHV1 and AciHV2 are effectively transmitted through water, and farms which share a common water source with an infected population may be exposed.
- The likelihood of AciHV1 and AciHV2 spread from farms to neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however AciHV1 and AciHV2 could spread this way. AciHV1 and AciHV2 could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.
- It is expected that AciHV1 and AciHV2 could not establish in the wild as there are no known wild sturgeon populations in Australian waters.

### Conclusion

Based on these considerations and using the descriptors in Table 4, the partial likelihood of establishment and spread of AciHV1 and AciHV2 in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

### Determining adverse impacts resulting from the outbreak scenario

The key points relevant when determining the adverse impacts resulting from establishment and spread of AciHV1 and AciHV2 were:

#### *Direct effects*

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

- AciHV1 and AciHV2 are only known to infect species of sturgeon. There is high morbidity and mortality associated with infection. Therefore, the establishment of a sturgeon industry in Australia would be significantly affected by an outbreak. AciHV1 and AciHV2 are not expected to affect any other species of farmed fish in Australia.
- AciHV1 and AciHV2 are not expected to impact wild fisheries in Australia. AciHV1 and AciHV2 have only been found in sturgeon species.
- Based on the impact of AciHV1 and AciHV2 on commercial sturgeon farms, the establishment and spread of AciHV1 and AciHV2 in Australia would be expected to cause minor impacts at the state or territory 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*

- AciHV1 and AciHV2 are only known to infect sturgeon and there are no known wild populations present in Australia.



- The direct impact of AciHV1 and AciHV2 on the living environment is not expected to be discernible at any level.

*Indirect effects*

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

- Infection with sturgeon alloherpesviruses is not listed as a notifiable disease by WOA and is not included on Australia's *National list of reportable diseases of aquatic animals*. Although not listed in Australia, state and territory governments would be expected to report on the presence of an unlisted agent that has never been reported in Australia.
- If AciHV1 and AciHV2 were confirmed at a farm, then attempts at eradication would likely 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.
- Eradication and control of AciHV1 and AciHV2 is expected to cause minor impacts at the state or territory 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*

- 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.
- Infected fish may show clinical signs which would affect their marketability. AciHV1 and AciHV2 infections would affect caviar production.
- AciHV1 and AciHV2 establishment and spread would likely have a minor impact at the local 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 sturgeon alloherpesviruses is not a WOA-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to sturgeon alloherpesviruses.
- However, if countries were to impose import requirements for AciHV1 and AciHV2, and the hazards were to become established, Australia could use zoning to maintain or gain access to international markets for live sturgeons, and if required, non-viable sturgeon products.
- The impacts of AciHV1 and AciHV2 establishment and spread on international trade are likely to be minor at the local level.

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

- AciHV1 and AciHV2 are only known to infect species of sturgeon and there are no known endangered Australian species, or closely related species, susceptible to AciHV1 and AciHV2.

- The impact of AciHV1 and AciHV2 establishment and spread on the biodiversity of the environment 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*

- AciHV1 and AciHV2 are only known to infect species of sturgeon and there are no known wild populations in Australia. Therefore, AciHV1 and AciHV2 would not affect any species recreationally fished in Australia.
- In local areas where a sturgeon aquaculture industry is established, AciHV1 and AciHV2 outbreaks could cause loss of business and welfare concerns.
- The social impacts of AciHV1 and AciHV2 establishment and spread are expected to be minor at the local level.

Table 25 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of AciHV1 and AciHV2. 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 25 Overall impact of establishment and spread of AciHV1 and AciHV2 for the outbreak scenario**

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	State or territory	Minor	D
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	State or territory	Minor	D
	Economic (domestic trade effects and impact on other associated industries)	Local	Minor	B
	Economic (international trade effects)	Local	Minor	B
	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)	Local	Minor	B

### Conclusion

The overall impact of establishment and spread of AciHV1 and AciHV2 was estimated to be **low**.

### 16.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for AciHV1 and AciHV2 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Negligible**.

### 16.3.6 Determination of the partial annual risk

The partial annual risk of AciHV1 and AciHV2 entry, establishment and spread from **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Negligible**.

The partial annual risk of AciHV1 and AciHV2 entry, establishment and spread from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Negligible**.

### 16.3.7 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 risk associated with AciHV1 and AciHV2 was found to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

Therefore, as the overall annual risk does not achieve Australia's ALOP for AciHV1 and AciHV2, specific biosecurity measures are considered necessary for this hazard.

## 16.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for AciHV1 and AciHV2 in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 16.4.1 Biosecurity measures that on their own achieve Australia's ALOP

#### Sourced from disease-free stocks

When determining if sourcing live sturgeon or their reproductive material from a stock recognised by the department as free of AciHV1 and AciHV2 would reduce the likelihood of entry, the factors considered were:

- AciHV1 and AciHV2 are found in North America and Europe (Hedrick et al. 1991b; LaPatra et al. 2014; Shchelkunov et al. 2009; Watson et al. 1995).
- AciHV1 and AciHV2 freedom would need to be assessed to a standard consistent with that recommended for World Organisation for Animal Health (WOAH) listed diseases, or equivalent.

Sourcing from disease-free stocks alone would be sufficient to reduce the likelihood of entry of AciHV1 and AciHV2 in **imported live sturgeon** or their **reproductive material** from **high** to **very low**. This reduces the overall annual restricted risk of live sturgeon or their reproductive material to **negligible**, thereby achieving Australia's ALOP.

### 16.4.2 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 26 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of AciHV1 and AciHV2 but which on their own do not achieve Australia's ALOP for AciHV1 and AciHV2 in imported live sturgeon or their reproductive material.

**Table 26 Biosecurity measures that on their own do not achieve Australia's ALOP for AciHV1 and AciHV2**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Post-arrival quarantine (PAQ)	<b>Yes:</b> The stress of transport can induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
2	Post-arrival batch testing	<b>Yes:</b> There are PCR methods available to detect AciHV1 and AciHV2 (Dospoly et al. 2008; Dospoly & Shchelkunov 2010; Kelley et al. 2005; Kurobe et al. 2008; Waltzek et al. 2009). Under this scenario, testing is conducted under departmental control and oversight.

### 16.4.3 Biosecurity measures that in combination achieve Australia's ALOP

A combination of biosecurity measures 1 and 2 when applied to **imported live sturgeon** would reduce the likelihood of entry of AciHV1 and AciHV2 from **high** to **very low**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1 and 2 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of AciHV1 and AciHV2 from **high** to **very low**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

# 17 Sturgeon nucleocytoplasmic large DNA viruses

## 17.1 Background

Sturgeon nucleocytoplasmic large DNA viruses (sNCLDV) can cause lethal disease in sturgeon (Mugetti et al. 2020b). sNCLDV currently includes six viruses (Mugetti et al. 2020b):

- Acipenser iridovirus-European (AcIV-E)
- British Columbia white sturgeon virus (BCWSV)
- Namao virus (NV)
- Missouri River sturgeon iridovirus (MRSIV)
- shortnose sturgeon virus (SNSV)
- white sturgeon iridovirus (WSIV).

Serious losses in farmed sturgeon due to sNCLDV were first reported with WSIV infections in the United States of America (USA) in the 1980s (Hedrick et al. 1990). sNCLDV have since been detected in North America and Europe in both wild and farmed sturgeon (Bigarré et al. 2017; Hedrick et al. 1990; Hofsoe-Oppermann et al. 2019; LaPatra et al. 1994; Mugetti et al. 2020a; Mugetti et al. 2020b; Raverty et al. 2003; Rud et al. 2020).

Infection with sNCLDV is not listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (OIE 2020). Infection with WSIV was previously listed by WOAH but has since been removed. Infection with sNCLDV is not included on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). sNCLDV are considered exotic to Australia.

## 17.2 Technical information

### 17.2.1 Agent properties

sNCLDV are a group of icosahedral, enveloped, double-stranded DNA viruses that vary in size (Mugetti et al. 2020b). BCWSV are 261–305 nm in diameter (Raverty et al. 2003), MRSIV are 250–260 nm (Kurobe et al. 2011), NV are 242–282 nm (Clouthier et al. 2013), SNSV are 176–196 nm (LaPatra et al. 2014), and WSIV are 262–273 nm (Hedrick et al. 1990; Hedrick et al. 1992).

Nucleocytoplasmic large DNA viruses are problematic as members infect a wide range of eukaryotes but few have been isolated or studied compared to other viruses (Meng et al. 2021). sNCLDV includes members previously identified as Iridovirus by electron microscopy but which are now not recognised as members of the *Iridoviridae* (Hedrick et al. 1992; Kurobe et al. 2011). They have not yet been formerly assigned by the International Committee on Taxonomy of Viruses to any taxonomic family but phylogenetic analysis of the major capsid protein suggests they could be classified as members of the *Mimiviridae* family (Clouthier et al. 2018). *Mimiviridae* are the most abundant group of viruses in the marine environment, over 5000 members can be detected in a few litres of seawater (Li et al. 2018).

MRSIV replicates at 11–15°C (Kurobe et al. 2011). WSIV replicates at 10–20°C but not at 25°C (Hedrick et al. 1992). AcIV-E was identified at water temperatures of 12–20°C (Bigarré et al. 2017). Water temperature may also affect the time from infection to appearance of clinical signs. For example, at water temperatures of 23°C, 19°C, 14°C and 10°C, the incubation period until clinical signs due to WSIV appeared were 7, 10, 20 and 40 days, respectively (Watson, Milani & Hedrick 1998).

There is little information on the stability or inactivation of sNCLDV. WSIV and MSIV survive freezing as virus stocks and infected dead fish stored at –70°C and –80°C were used to induce infections in healthy sturgeon (Hedrick et al. 1992; Kurobe et al. 2011; Kwak et al. 2006). WSIV could be partially inactivated following incubation at 56°C for 30 minutes with 0.1% infectivity remaining (Hedrick et al. 1992).

## 17.2.2 Epidemiology

### Host range

Species which are reported to be susceptible to infection with sNCLDV (N= natural exposure; E= experimental exposure) include but are not limited to:

- *Acipenser baerii*<sup>N</sup> (Siberian sturgeon)
  - Infected with AcIV-E (Bigarré et al. 2017; Ciulli et al. 2016)
  - Infected with WSIV (Hofsoe-Oppermann et al. 2019)
- *Acipenser brevirostrum*<sup>N</sup> (shortnose sturgeon)
  - Infected with SNSV (LaPatra et al. 2014)
- *Acipenser fulvescens*<sup>N,E</sup> (lake sturgeon)
  - Infected with NV (Clouthier et al. 2013)
  - Infected with WSIV (Hedrick et al. 1992)
- *Acipenser gueldenstaedtii*<sup>N</sup> (Russian sturgeon)
  - Infected with AcIV-E (Bigarré et al. 2017; Ciulli et al. 2016; Pallandre et al. 2018)
  - Infected with WSIV (Hofsoe-Oppermann et al. 2019)
- *Acipenser naccarii*<sup>N</sup> (Adriatic sturgeon)
  - Infected with AcIV-E (Bigarré et al. 2017)
- *Acipenser oxyrinchus*<sup>N</sup> (Atlantic sturgeon)
  - Infected with WSIV (Hofsoe-Oppermann et al. 2019, 2020)
- *Acipenser ruthenus*<sup>N</sup> (sterlet sturgeon)
  - Infected with AcIV-E (Mugetti et al. 2020a)
- *Acipenser stellatus*<sup>N</sup> (starry sturgeon)
  - Infected with AcIV-E (Mugetti et al. 2020a)
- *Acipenser transmontanus*<sup>N,E</sup> (white sturgeon)

- Infected with BCWSV (Raverty et al. 2003)
- Infected with WSIV (Hedrick et al. 1990; LaPatra et al. 1994)
- *Huso huso*<sup>N</sup> (Beluga sturgeon)
  - Infected with AcIV-E (Bigarré et al. 2017)
- *Scaphirhynchus albus*<sup>N, E</sup> (pallid sturgeon)
  - Infected with MRSIV (Kurobe et al. 2011)
- *Scaphirhynchus platyrhynchus*<sup>N, E</sup> (shovelnose sturgeon)
  - Infected with MRSIV (Kurobe et al. 2011).

sNCLDV-positive PCR results have been reported in a few species (N= natural exposure; E= experimental exposure) however no active infection has been demonstrated, including:

- *Acipenser ruthenus*<sup>N</sup> (sterlet sturgeon)
  - Detection of WSIV (Hofsoe-Oppermann et al. 2019)
- *Acipenser sturio*<sup>N</sup> (European sea sturgeon)
  - Detection of WSIV (Hofsoe-Oppermann et al. 2019).

AcIV-E and MRSIV infects juvenile and adult sturgeon (Bigarré et al. 2017; Ciulli et al. 2016; Kurobe et al. 2010; Kurobe et al. 2011; Mugetti et al. 2020a). BCWSV was detected in fingerling *A. transmontanus* 8–10 cm length (Raverty et al. 2003). NV was identified in juvenile *A. fulvescens* (mean weight 3–19.3 g) (Clouthier et al. 2013). SNSV affected *A. brevirostrum* of mean weight 800 g (LaPatra et al. 2014). WSIV was detected in fry, fingerlings, juveniles and fish up to 50 cm (Hedrick et al. 1990; Mugetti et al. 2020b).

### Geographical distribution

AcIV-E was found in France (Pallandre et al. 2018), Italy (Ciulli et al. 2016), Poland (Stachnik et al. 2021), Sweden (Axen, Vendramin & Toffan 2018) and Ukraine (Rud et al. 2020). MRSIV has been detected in the USA (Kurobe et al. 2011). BCWSV, NV and SNSV were reported in Canada (Clouthier et al. 2013; LaPatra et al. 2014; Raverty et al. 2003). WSIV was reported from the USA (Hedrick et al. 1990; LaPatra et al. 1994), Germany, Italy and Poland (Hofsoe-Oppermann et al. 2019).

### Prevalence

Screening of *A. baerii* (weight 10–2000 g; n=120) and *A. gueldenstaedtii* (weight 10–1500 g; 16 fish) collected from 9 farms in Poland in 2016–2020 identified AcIV-E at a prevalence of 26% (n=136) (Stachnik et al. 2021).

MRSIV prevalence of 83% (n=53) was detected in wild adult *S. platyrhynchus* captured from the Missouri River, USA in 2006 (Kurobe et al. 2010).

PCR-positive results for WSIV were obtained in 92.62% (n=244) of tissue samples collected from *A. baerii*, *A. gueldenstaedtii*, *A. oxyrinchus*, *A. ruthenus* and *A. sturio* (n=79) in 2010–2014 from hatcheries in Germany, Italy and Poland (Hofsoe-Oppermann et al. 2019). Monitoring of progeny at a *A. transmontanus* hatchery in Northern California, USA, during 1997–1998 detected 5 WSIV outbreaks during 6 spawns (Georgiadis et al. 2001). WSIV was detected in 85% (n=54) of *A. transmontanus* grow out tanks from 3 farms in California, USA during a study conducted between

1997–1998 (Georgiadis et al. 2000). WSIV was also found in wild healthy *A. gueldenstaedtii* (n=1) and *A. oxyrinchus* (n=4) collected from open waters in Poland between 2010–2014 (Hofsoe-Oppermann et al. 2020).

A sNCLDV prevalence of 8.7% (n=69) was reported from *A. fulvescens* broodstock collected from the Nelson River and Winnipeg River drainage systems in Manitoba, Canada during 2010–2013 (Clouthier, VanWalleghem & Anderson 2015). Additionally, 26% (n=640) of the hatchery-reared juveniles originating from the same wild broodstock later tested positive for sNCLDV (Clouthier, VanWalleghem & Anderson 2015).

No prevalence data was found for BCWSV, NV and SNSV.

### **Mortalities**

*A. gueldenstaedtii* juveniles farmed in a European country (name not disclosed) infected with AcIV-E during 2014–2015 suffered mortalities of 30–100% (Bigarré et al. 2017). An AcIV-E outbreak in *A. gueldenstaedtii* and *A. baerii* fingerlings on a farm in Italy in 2015 caused mortalities of 90% and 50%, respectively, though the sturgeon also had concurrent bacterial infections that may have contributed to the mortality (Ciulli et al. 2016). In 2017, AcIV-E-infected *A. baerii* juveniles imported to a Swedish farm reached a cumulative mortality of 95% 2 months after arrival (Axen, Vendramin & Toffan 2018). An outbreak of AcIV-E in *A. stellatus* on a farm in Italy in 2018 resulted in cumulative mortalities of 25% over 9–10 months (Mugetti et al. 2020a). Infected *A. ruthenus* on the same farm exhibited 20% mortality and infected *A. gueldenstaedtii* had mortalities of 25–90% (Mugetti et al. 2020a).

An MRSIV outbreak in 1999 in a *S. platyrhynchus* hatchery on the Missouri River, USA that was stocked with juveniles spawned from wild broodstock caused up to 100% mortality in some groups (Kurobe et al. 2011). A subsequent outbreak of MRSIV in juvenile *S. albus* at a second hatchery caused 60% mortality (Kurobe et al. 2011).

NV outbreaks in hatchery-reared juvenile *A. fulvescens* occurred in Manitoba, Canada in 2009–2010 causing cumulative mortalities of 62–99.6% (Clouthier et al. 2013).

*A. brevirostrum* in a hatchery in Canada infected with SNSV suffered mortality but no numbers were provided (LaPatra et al. 1994).

A farm in California, USA with *A. transmontanus* 200,000 juveniles infected with WSIV suffered 95% mortality over a 4-month period (Hedrick et al. 1990). Juvenile losses in a *A. transmontanus* hatchery in Oregon, USA in 1990 were approximately 23% (LaPatra et al. 1994). Mortalities in *A. transmontanus* juveniles due to a WSIV outbreak in hatcheries in Idaho, USA in 1992 reached 52% (LaPatra et al. 1994).

### **Transmission**

Experimentally, MRSIV and WSIV can be transmitted by cohabitation with infected fish and by bath exposure with virus extracts and tissue homogenates (Drennan et al. 2006; Hedrick et al. 1990; Hedrick et al. 1992). MRSIV infections have also been induced by feeding infected *S. platyrhynchus* tissues to healthy *S. albus* (Kurobe et al. 2011). AcIV-E can likely be transmitted by water as the virus was transmitted from infected *A. gueldenstaedtii* to healthy *A. ruthenus* and *A. stellatus* held separately but reared on the same farm (Mugetti et al. 2020a). Transmission of WSIV from



broodstock to progeny has been suggested to occur due to the detection of infected progeny from wild-caught broodstock but vertical transmission has not been definitively demonstrated (Drennan et al. 2006; Georgiadis et al. 2001; Hedrick et al. 1992).

There is considerable evidence for a sNCLDV carrier state in sturgeon. For example, AcIV-E was detected in naturally infected *A. gueldenstaedtii* survivors up to 4 months after the end of the outbreak (Ciulli et al. 2016). MRSIV was detected in naturally infected *S. albus* 5 months following the last mortality (Kurobe et al. 2011). Juvenile *S. albus* that had recovered from MRSIV clinical disease were shown to harbor viral DNA for up to 8.5 months, and when cohabitated with healthy sturgeon, were able to transmit MRSIV and induce disease (Kurobe et al. 2011). WSIV infections in hatcheries are thought to have originated from wild sturgeon adults collected for broodstock (Hedrick et al. 1990). WSIV could be detected by PCR in surviving *A. transmontanus* 9 months post infection (Kwak et al. 2006).

Stress factors such as transportation, rearing density, handling, water quality and fluctuations in water temperature, levels and flow rates may affect the onset and severity of sNCLDV outbreaks (Drennan et al. 2005; Drennan et al. 2006; Georgiadis et al. 2001; LaPatra et al. 1994). Co-infection of sturgeon with multiple sNCLDV or bacteria can also occur which would be another stress factor that could induce clinical disease (Bigarré et al. 2017; Ciulli et al. 2016; Raverty et al. 2003; Stachnik et al. 2021).

The spread of sNCLDV into new areas is likely attributed to the movement of live animals. For example, an outbreak of AcIV-E in farmed *A. baerii* in Sweden was attributed to the importation of juveniles from Italy (Axen, Vendramin & Toffan 2018). For outbreaks of WSIV on *A. transmontanus* farms in Oregon and Idaho, USA, it was suspected that WSIV entered the facilities via river water or eggs from wild broodstock (Georgiadis et al. 2000).

### Infectious dose

Bath exposure of *S. albus* (mean weight 7.9 g) to  $10^{4.0}$ ,  $10^{3.1}$  and  $10^{1.9}$  MRSIV copies/L for 1 hour resulted in cumulative mortalities of 81%, 69% and 85%, respectively, at 85 dpi (Kurobe et al. 2011). *S. albus* (mean weight 12 g) challenged by immersion with crude ( $10^{7.3}$  copies/mL) or filtered ( $10^{7.0}$  copies/mL) MRSIV extracts from virus-infected tissues for 1 hour suffered mortality from 39 dpi that reached 70% (Kurobe et al. 2011). MRSIV-exposed *S. albus* (mean weight 7.9 g) that survived experimental exposure and which had a mean viral concentration of  $10^{2.6}$  copies/ $\mu$ g DNA were cohabited for 2 weeks with healthy *S. albus* (mean weight 25 g) and induced infection and mortalities up to 65% (Kurobe et al. 2011).

Bath exposure of juvenile *A. transmontanus* (weight 6.4 g) with WSIV at  $10^3$  TCID<sub>50</sub>/g of fish for 30 minutes induced clinical signs at 10 days post infection (dpi) and 80% cumulative mortality by 50 dpi (Hedrick et al. 1992). Bath exposure of *A. fulvescens* (weight 4.5 g) to the same WSIV concentration induced infection but no mortality (Hedrick et al. 1992). A WSIV bath challenge dose of 500 TCID<sub>50</sub>/mL ( $10^{4.3}$  TCID<sub>50</sub>/g of fish) for 45 minutes was sufficient to initiate infection in *A. transmontanus* (mean weight 0.6 g) with clinical signs observed at 10–14 dpi (Watson, Groff & Hedrick 1998). Bath exposure of *A. transmontanus* (mean weight 1.75 g) with WSIV at  $10^{3.5}$  TCID<sub>50</sub>/g of fish for 1 hour and grown out at temperatures ranging from 10–23°C caused cumulative mortalities ranging from 71% to 54% from the lowest to the highest water temperature (Watson, Milani & Hedrick 1998).

There were no reports on infectious doses for AcIV-E, BCWSV, NV or SNSV.

### 17.2.3 Pathogenesis

#### Tissue tropism

sNCLDV infect epithelial cells of the skin, gills, fins, barbels, oropharynx, nasal organ, operculum, rostrum and cranium (Bigarré et al. 2017; Ciulli et al. 2016; Clouthier et al. 2013; Hedrick et al. 1990; Kurobe et al. 2010; Kurobe et al. 2011; LaPatra et al. 2014; Raverty et al. 2003; Watson, Groff & Hedrick 1998). AcIV-E and WSIV have also been detected in the kidney, liver, spleen and brain (Ciulli et al. 2016; Hofsoe-Oppermann et al. 2020).

#### Tissue titre

The MRSIV concentration in pectoral and anal fins from bath challenged *S. albus* at 69 dpi ranged from  $10^{4.4}$ – $10^{4.8}$  copies/μg DNA (Kurobe et al. 2011). The mean MRSIV concentrations in infected *S. albus* 8.5 months post exposure were  $10^{2.3}$  copies/μg DNA in pectoral fins,  $10^{3.9}$  copies/μg DNA in anal fins and  $10^{3.2}$  copies/μg DNA in barbel (Kurobe et al. 2011). The MRSIV mean concentration in pelvic fins of *S. albus* following cohabitation with infected sturgeon was  $10^{6.2}$  copies/μg in dead fish and  $10^{3.5}$  copies/μg DNA in survivor fish at 54 dpi (Kurobe et al. 2011). Infected *S. platyrhynchus* reported a MRSIV titre that ranged from  $10^{0.5}$ – $10^{4.5}$  copies/μg DNA in the pectoral fins (Kurobe et al. 2010).

Dead *A. transmontanus* (weight 6.4 g) bath exposed to WSIV had virus concentrations of  $10^{4.5}$ – $10^5$  TCID<sub>50</sub>/g (Hedrick et al. 1992).

There were no reports on tissue titres for AcIV-E, BCWSV, NV or SNSV.

### 17.2.4 Diagnosis

#### Clinical signs

The clinical signs of sNCLDV infection include lethargy, cessation of feeding, pale colour, skin ulcers, respiratory distress, petechial haemorrhages on skin and gills, excess gill mucus, erratic swimming and fish typically fall to the bottom of the tank prior to death (Bigarré et al. 2017; Ciulli et al. 2016; Clouthier et al. 2013; Hedrick et al. 1990; Kurobe et al. 2011; LaPatra et al. 2014; Mugetti et al. 2020b; Raverty et al. 2003). In some cases, particularly in adults, no clinical signs are seen (Bigarré et al. 2017; Ciulli et al. 2016; Georgiadis et al. 2001; Hofsoe-Oppermann et al. 2019; Kurobe et al. 2010; Stachnik et al. 2021).

#### Pathology

Histopathology of sNCLDV-infected sturgeon show hypertrophied epithelial cells in the skin, gills, oropharynx, fins, barbels, rostrum and cranium with basophilic or amphophilic- to eosinophilic-staining and eccentric nuclei (Bigarré et al. 2017; Ciulli et al. 2016; Clouthier et al. 2013; Hedrick et al. 1990; Kurobe et al. 2011; LaPatra et al. 2014; Raverty et al. 2003; Watson, Milani & Hedrick 1998). Pale kidneys, liver and spleen, degeneration and necrosis of epidermal cells, fatty liver and catarrhal enteritis were also observed (Bigarré et al. 2017; Ciulli et al. 2016; Hedrick et al. 1990; LaPatra et al. 2014; Rud et al. 2020).

#### Testing

PCR and real-time PCR methods are available to detect AcIV-E (Bigarré et al. 2017; Ciulli et al. 2016), MRSIV (Kurobe et al. 2010), NV (Clouthier et al. 2013) and WSIV (Hofsoe-Oppermann et al. 2019; Kwak et al. 2006). Clouthier et al. (2015) developed a conventional PCR and real-time PCR based on

the genetic relatedness of the sNCLDV major capsid protein that detects BCWSV, MRSIV, NV, SNSV and WSIV (Clouthier, VanWalleghem & Anderson 2015).

sNCLDV can also be diagnosed by histopathology and electron microscopy (Bigarré et al. 2017; Ciulli et al. 2016; Clouthier et al. 2013; Hedrick et al. 1990; Hofsoe-Oppermann et al. 2019; Kurobe et al. 2011; LaPatra et al. 2014; Raverty et al. 2003). Only WSIV has been successfully isolated in cell culture (Hedrick et al. 1990).

### **17.2.5 Treatment**

There is no evidence of effective treatment methods for sNCLDV.

### **17.2.6 Control and prevention**

Good management practices such as screening of wild broodstock by PCR, sustaining low fish densities, using all-in, all-out production, maintaining virus-free water supplies, minimising adverse environmental conditions, disinfection of fomites and reducing the handling and stress of sturgeon younger than 1 year have been recommended to control and prevent WSIV and MRSIV infections and could be applied to all sNCLDV infections (Georgiadis et al. 2000; Kurobe et al. 2011; LaPatra et al. 1994). It is unknown if iodophor disinfection of eggs would prevent sNCLDV transmission (Drennan et al. 2006).

### **17.2.7 Impact of the disease**

WSIV is considered one of the two most important viruses limiting the survivability of juvenile sturgeon (Georgiadis et al. 2000). Although, all sNCLDV can cause serious mortalities in sturgeon. As WSIV is primarily a disease of juveniles, its effects are most significant in the hatchery. There are no reports on the economic losses in sturgeon aquaculture due to sNCLDV.

### **17.2.8 Current biosecurity measures**

There are no biosecurity measures for live sturgeon and their reproductive material as import is not permitted.

## **17.3 Risk assessment**

Based on [chapter 4](#) and the technical information about sNCLDV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of sNCLDV meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### **17.3.1 Entry assessment**

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that sNCLDV are present in all source countries.
- sNCLDV infects sturgeon life stages that would be exported to Australia.
- Prevalence of sNCLDV in farmed sturgeon is variable but can be up to 85%.
- There are reports of sNCLDV in wild *A. gueldenstaedtii*, *A. oxyrinchus* and *S. platorynchus* (Hofsoe-Oppermann et al. 2020; Kurobe et al. 2010).

- There are no reports of sNCLDV associating with sturgeon reproductive material though egg-associated transmission has been suggested.
- It is unknown if sNCLDV can remain viable outside the host.
- The viral load of sNCLDV in infected imported live sturgeon or their reproductive material is likely to be sufficient to cause infection in susceptible species.
- Inspection may detect sturgeon showing clinical signs of infection with sNCLDV and remove them before export. Sturgeon subclinically infected or carrier fish would not be identified through visual inspection.
- Sturgeon reproductive material infected or contaminated with sNCLDV are unlikely to be detected during inspection because there would be no clinical signs.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of sNCLDV was estimated to be:

- Imported live sturgeon—**High**.
- Imported sturgeon reproductive material—**High**.

### 17.3.2 Exposure assessment

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

- sNCLDV can be transmitted horizontally via water and ingestion of infected tissue.
- sNCLDV would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- Only sturgeon species are susceptible to sNCLDV.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are not susceptible to sNCLDV.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the range of 10–20°C when sturgeon are susceptible to sNCLDV (Bigarré et al. 2017; Hedrick et al. 1992; Kurobe et al. 2011).
- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infected sturgeon will be certain to be exposed to viable sNCLDV.
- Introduction into the wild may occur by direct release of imported live sturgeon. Release of contaminated associated wastes from the aquaculture facility into natural waters would also be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for waste management that would exclude sNCLDV from discharges. However, there are no known wild susceptible species of sNCLDV present in Australia to be exposed.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to sNCLDV in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to sNCLDV in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

### 17.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to sNCLDV in **imported live sturgeon** 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:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

The partial annual likelihood of entry and exposure of each exposure group to sNCLDV in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

### 17.3.4 Consequence assessment

#### Partial likelihood of establishment and spread

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

- sNCLDV can be transmitted horizontally via water and ingestion of infected tissue. Transmission from broodstock to progeny may also occur.
- It is unknown how long sNCLDV can remain viable outside the host.
- It is expected that susceptible host animals in contact with sNCLDV-infected sturgeon would receive an infectious dose.
- Sturgeon that survive sNCLDV infection can remain infectious and become sources of the virus.
- Outbreaks of sNCLDV are typically observed at water temperatures of 10–20°C.
- Only sturgeon species are susceptible to sNCLDV, which are not yet found in Australia.
- The likelihood of sNCLDV establishment, following a given quantity of sNCLDV entering the environment of an exposure group, is greatest for farmed susceptible species. This is due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- If infected live sturgeon or their reproductive material were moved to another aquaculture facility in Australia it is likely that sNCLDV would establish in these facilities.

- Each state and territory have translocation protocols for aquaculture animals but are unlikely to include consideration of sNCLDV. Although, WSIV is notifiable in South Australia.
- If sNCLDV were to establish on a farm it could spread to neighbouring farms and wild waters through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of sNCLDV be suspected and response measures initiated immediately. However, sNCLDV is effectively transmitted through water and farms which share a common water source or equipment with an infected population may be exposed to sNCLDV.
- The likelihood of sNCLDV spread from farms to wild waters or neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however sNCLDV could spread this way. sNCLDV could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.
- If one or more index cases of sNCLDV were to occur in the wild, establishment and spread is unlikely as there are no known wild susceptible species in Australia. The ability of fish to be subclinically infected with sNCLDV and to remain carriers after surviving an infection would however aid its spread to other sturgeon if they were present in the wild.

### Conclusion

Based on these considerations and using the descriptors in Table 4, the partial likelihood of establishment and spread of sNCLDV in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**High**.
- Wild susceptible species—**Negligible**.

### Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of sNCLDV were that:

#### *Direct effects*

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

- sNCLDV are only known to infect species of sturgeon. There is high morbidity and mortality associated with infection. Therefore, the establishment of a sturgeon industry in Australia would be significantly affected by an outbreak of sNCLDV.
- sNCLDV is not expected to have an impact on other species of farmed fish in Australia.
- sNCLDV is not expected to impact wild fisheries in Australia. sNCLDV has only been found in sturgeon species.
- Based on the impacts of sNCLDV on sturgeon farms in the Americas and Europe, sNCLDV establishment and spread in Australia would be expected to cause minor impacts at the state or territory 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*

- sNCLDV are only known to infect sturgeon and there are no wild populations present in Australia.
- The direct impact of sNCLDV on the living environment is not expected to be discernible at any level.

*Indirect effects*

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

- Infection with sNCLDV is not listed as a notifiable disease by WOA and is not included on Australia's *National list of reportable diseases of aquatic animals*. Although not listed in Australia, state and territory governments would be expected to report on the presence of an unlisted agent that has never been reported in Australia. WSIV is a notifiable disease in South Australia.
- If sNCLDV were confirmed at a farm, then attempts at eradication would likely 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.
- Eradication and control of sNCLDV is expected to cause minor impacts at the state or territory 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*

- 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.
- sNCLDV-infected fish may show clinical signs which would affect their marketability. sNCLDV infections would affect caviar production.
- Interstate trade in infected sturgeon may be impacted during an outbreak, given WSIV is a notifiable disease in South Australia.
- sNCLDV establishment and spread would likely have a minor impact at the local 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*

- sNCLDV is not a WOA-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to sNCLDV to avoid the possible introduction of sNCLDV.
- If sNCLDV were to become established, Australia could use zoning to maintain or gain access to international markets for live sturgeon and non-viable finfish products.
- The impacts of sNCLDV establishment and spread on international trade are likely to be minor at the local level.

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

- sNCLDV is only known to infect species of sturgeon and there are no known endangered Australian species, or closely related species, susceptible to sNCLDV.
- The impact of sNCLDV establishment and spread on the biodiversity of the environment 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*

- sNCLDV are only known to infect species of sturgeon and there are no known susceptible species in the wild. Therefore, it is expected that sNCLDV would not affect any species recreationally fished in Australia.
- In local areas where a sturgeon aquaculture industry is established, a sNCLDV outbreak could cause loss of business and welfare concerns.
- The social impacts of sNCLDV establishment and spread are expected to be minor at the local level.

Table 27 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of sNCLDV. 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 27 Overall impact of establishment and spread of sNCLDV for the outbreak scenario**

Effects	Criteria	Level	Impact	Score
Direct	Animal health (production losses in aquaculture and commercial fisheries)	State or territory	Minor	D
	The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernible	A
Indirect	Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	State or territory	Minor	D
	Economic (domestic trade effects and impact on other associated industries)	Local	Minor	B
	Economic (international trade effects)	Local	Minor	B
	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)	Local	Minor	B

### Conclusion

The overall impact of establishment and spread of sNCLDV was estimated to be **low**.

### 17.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for sNCLDV 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Negligible**.



### 17.3.6 Determination of the partial annual risk

The partial annual risk of sNCLDV entry, establishment and spread from **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Negligible**.

The partial annual risk of sNCLDV entry, establishment and spread from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species— **Low**.
- Wild susceptible species—**Negligible**.

### 17.3.7 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 sNCLDV was found to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

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

## 17.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for sNCLDV in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 17.4.1 Biosecurity measures that on their own achieve Australia's ALOP

#### Sourced from disease-free stocks

When determining if sourcing live sturgeon or their reproductive material from a stock recognised by the department as free of sNCLDV would reduce the likelihood of entry, the factors considered were:

- sNCLDV is found in North America and Europe (Bigarré et al. 2017; Hedrick et al. 1990; Hofsoe-Oppermann et al. 2019; LaPatra et al. 1994; Mugetti et al. 2020a; Mugetti et al. 2020b; Raverty et al. 2003; Rud et al. 2020).
- sNCLDV freedom would need to be assessed to a standard consistent with that recommended for World Organisation for Animal Health (WOAH) listed diseases, or equivalent.

Sourcing from disease-free stocks alone would be sufficient to reduce the likelihood of entry of sNCLDV in **imported live sturgeon** or their **reproductive material** from **high** to **very low**. This reduces the overall annual restricted risk of live sturgeon or their reproductive material to **negligible**, thereby achieving Australia's ALOP.

### 17.4.2 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 28 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of sNCLDV but which on their own do not achieve Australia's ALOP for sNCLDV in imported live sturgeon or their reproductive material.

**Table 28 Biosecurity measures that on their own do not achieve Australia's ALOP for sNCLDV**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Post-arrival quarantine (PAQ)	<b>Yes:</b> The stress of transport can induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
2	Post-arrival batch testing	<b>Yes:</b> There are PCR and real-time PCR methods available to detect sNCLDV (Clouthier, VanWalleghem & Anderson 2015). Under this scenario, testing is conducted under departmental control and oversight.

### 17.4.3 Biosecurity measures that in combination achieve Australia's ALOP

A combination of biosecurity measures 1 and 2 when applied to **imported live sturgeon** would reduce the likelihood of entry of sNCLDV from **high** to **very low**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1 and 2 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of sNCLDV from **high** to **very low**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

# 18 Viral haemorrhagic septicaemia virus

## 18.1 Background

Viral haemorrhagic septicaemia virus (VHSV), also known as *Novirhabdovirus piscine*, is the aetiological agent of viral haemorrhagic septicaemia (VHS) (Ghittino 1965; WOA 2022c). VHS is a serious disease that causes high mortalities of both farmed and wild fish in freshwater and marine environments (WOAH 2022c). 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 *O. mykiss* (Skall, Olesen & Møllergaard 2005b). In the 1950s, the syndrome appeared in Danish freshwater *O. mykiss* farms and a virus aetiology was confirmed (Jensen 1965). Infections with VHSV have since been detected in at least 30 countries in North Asia, North America and Europe (CEFAS 2022; WOA 2022c).

Infection with VHSV is listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (WOAH 2022c) and is listed on Australia's *National list of reportable diseases of aquatic animals* (Department of Agriculture 2020a). Australia has a long history of passive surveillance and a strong system in place to detect incursions. VHSV is considered exotic to Australia.

## 18.2 Technical information

### 18.2.1 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 2022c). 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 *O. mykiss* (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 *O. mykiss* 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. 2006b).

*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. 2013b; 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 *O. mykiss* and *Salmo salar* (Atlantic salmon) (Skall, Olesen & Møllergaard 2005b). In an immersion trial, a Norwegian isolate (*genotype III*) produced 70% mortality in *O. mykiss* whereas no mortality was observed in *S. salar* (Dale et al. 2009). Experimental infection of *O. mykiss* 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 2022c). 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 2022c).

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 2022c)). Hawley & Garver (2008) reported that in raw fresh water, the 99% inactivation time of 4 VHSV isolates ranged from 40 days at 4°C to less than 1 day at 30°C (Hawley & Garver 2008). The 99% inactivation time of the isolates in filtered fresh water ranged from >489 days at 4°C to <2 days at 30°C. When the viral isolates were incubated in raw sea water, 99.9% inactivation ranged from 13 days at 4°C to 1.5 days at 20°C. In filtered sea water, the 99.9% inactivation times ranged from 8.7 days at 4°C to 0.5 days at 20°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; WOAHA 2022c).

### 1.1.1 Epidemiology

#### Host range

Species which fulfil the criteria for listing as a species susceptible to infection (N=natural exposure; E=experimental exposure) with VHSV in accordance with chapter 1.5 of the WOAHA Code (WOAHA 2022b) include:

- *Alosa immaculata*<sup>N</sup> (pontic shad) (Ogut & Altuntas 2014)
- *Ameiurus nebulosus*<sup>N</sup> (brown bullhead) (Thompson et al. 2011)
- *Ammodytes hexapterus*<sup>N</sup> (Pacific sand lance) (Kocan et al. 2001)
- *Ambloplites rupestris*<sup>N</sup> (rock bass) (Thompson et al. 2011)
- *Aplodinotus grunniens*<sup>N</sup> (freshwater drum) (Lumsden et al. 2007)
- *Centrolabrus exoletus*<sup>N</sup> (rock cook wrasse) (Munro et al. 2015)
- *Clupea pallasii*<sup>N</sup> (Pacific herring) (Meyers et al. 1994)
- *Clupea harengus*<sup>N</sup> (Atlantic herring) (Dixon et al. 1997; Mortensen et al. 1999)
- *Coregonus artedii*<sup>N</sup> (lake cisco) (Thompson et al. 2011)
- *Coregonus clupeaformis*<sup>N</sup> (lake whitefish) (Thompson et al. 2011)
- *Coregonus lavaretus*<sup>E</sup> (common whitefish) (Skall, Kjær & Olesen 2004)
- *Coregonus species*<sup>N</sup> (whitefish) (Ahne & Thomsen 1985)
- *Ctenolabrus rupestris*<sup>N</sup> (goldsinny wrasse) (Munro et al. 2015)
- *Cyclopterus lumpus*<sup>N</sup> (lumpfish) (Gudmundsdóttir et al. 2019)
- *Cymatogaster aggregata*<sup>N, E</sup> (shiner perch) (Kent et al. 1998; Meyers & Winton 1995)
- *Danio rerio*<sup>E</sup> (zebra fish) (Novoa et al. 2006)
- *Dorosoma cepedianum*<sup>N</sup> (American gizzard shad) (USGS 2008)
- *Engraulis encrasicolus*<sup>N</sup> (European anchovy) (Ogut & Altuntas 2014)
- *Esox lucius*<sup>N</sup> (northern pike) ((Meier & Jorgensen 1979) cited in (Skall, Olesen & Mellergaard 2005b))

- *Esox masquinongy*<sup>N</sup> (muskellunge) (Elsayed et al. 2006; Faisal et al. 2012)
- *Fundulus heteroclitus*<sup>N</sup> (mummichog) (Olivier 2002)
- *Gadus aeglefinus*<sup>N</sup> (haddock) (Smail 2000)
- *Gadus macrocephalus*<sup>N</sup> (Pacific cod) (Meyers et al. 1992)
- *Gadus morhua*<sup>N</sup> (Atlantic cod) (Jensen & Larsen 1982)
- *Gaidropsarus vulgaris*<sup>N</sup> (three-bearded rockling) (Ogut & Altuntas 2014)
- *Gasterosteus aculeatus*<sup>N</sup> (three spined stickleback) (Kent et al. 1998)
- *Labrus bergylta*<sup>N</sup> (ballan wrasse) (Munro et al. 2015)
- *Labrus mixtus*<sup>N</sup> (cuckoo wrasse) (Munro et al. 2015)
- *Lampetra fluviatilis*<sup>N</sup> (river lamprey) (Gadd et al. 2010)
- *Lepomis gibbosus*<sup>N</sup> (pumpkinseed) (Faisal et al. 2012; Thompson et al. 2011)
- *Lepomis macrochirus*<sup>N</sup> (bluegill) (Faisal et al. 2012; USGS 2008)
- *Limanda limanda*<sup>N</sup> (common dab) (Skall, Olesen & Møllergaard 2005a)
- *Merlangius merlangus*<sup>N</sup> (whiting) (Mortensen et al. 1999)
- *Micromesistius poutassou*<sup>N</sup> (blue whiting) (Mortensen et al. 1999)
- *Micropterus dolomieu*<sup>N</sup> (smallmouth bass) (Faisal et al. 2012; USGS 2008)
- *Micropterus salmoides*<sup>N</sup> (largemouth bass) ((de Kinkelin et al. 1999) cited in (Skall, Olesen & Møllergaard 2005b))(Thompson et al. 2011)
- *Morone americana*<sup>N</sup> (white perch) (Thompson et al. 2011)
- *Morone chrysops*<sup>N</sup> (white bass) (Thompson et al. 2011)
- *Morone saxatilis*<sup>N</sup> (striped bass) (Gagné et al. 2007)
- *Mullus barbatus*<sup>N</sup> (red mullet) (Ogut & Altuntas 2014)
- *Neogobius melanostomus*<sup>N</sup> (round goby) (Groocock et al. 2007)
- *Notropis hudsonius*<sup>N</sup> (spottail shiner) (Faisal et al. 2012)
- *Notropis atherinoides*<sup>N</sup> (emerald shiner) (Faisal et al. 2012; USGS 2008)
- *Oncorhynchus aguabonita*<sup>E</sup> (golden trout) (Ahne, Negele & Ollenschlager 1976)
- *Oncorhynchus mykiss*<sup>N</sup> (rainbow trout) (Castric & de Kinkelin 1980; Jensen 1965)
- *Oncorhynchus mykiss* × *Oncorhynchus kisutch* hybrids<sup>E</sup> (rainbow trout × coho salmon hybrids) (Ord, Le Berre & de Kinkelin 1976)
- *Oncorhynchus kisutch*<sup>N</sup> (coho salmon) (Brunson, True & Yancey 1989)
- *Oncorhynchus tshawytscha*<sup>N</sup> (chinook salmon) (Hopper 1989)
- *Paralichthys olivaceus*<sup>N</sup> (Bastard halibut) (Isshiki et al. 2001; Takano et al. 2001)
- *Perca flavescens*<sup>N</sup> (yellow perch) (Kane-Sutton et al. 2010)
- *Pimephales notatus*<sup>N</sup> (bluntnose minnow) (Frattini et al. 2011)

- *Pimephales promelas*<sup>E</sup> (fathead minnow) (Al-Hussinee et al. 2010)
- *Platichthys flesus*<sup>N</sup> (European flounder) (Skall, Olesen & Møllergaard 2005a)
- *Pleuronectes platessius*<sup>N</sup> (European plaice) (Skall, Olesen & Møllergaard 2005a)
- *Pomatoschistus minutus*<sup>N</sup> (sand goby) (Skall, Olesen & Møllergaard 2005a)
- *Pomoxis nigromaculatus*<sup>N</sup> (black crappie) (Faisal et al. 2012; Thompson et al. 2011)
- *Raja clavata*<sup>N</sup> (thornback ray) (Ogut & Altuntas 2014)
- *Salmo marmoratus*<sup>E</sup> (marble trout) (Pascoli et al. 2015)
- *Salmo salar*<sup>E</sup> (Atlantic salmon) (de Kinkelin & Castric 1982)
- *Salmo trutta*<sup>N</sup> (brown trout) (Jørgensen 1980)
- *Salvelinus fontinalis*<sup>E</sup> (brook trout) (Rasmussen 1965)
- *Salvelinus namaycush*<sup>E</sup> (lake trout) (Dorson, Chevassus & Torhy 1991)
- *Sander vitreus*<sup>N</sup> (walleye) (Thompson et al. 2011)
- *Sardina pilchardus*<sup>N</sup> (pilchard) (Ogut & Altuntas 2014)
- *Sardinops sagax*<sup>N</sup> (South American pilchard, sardine) (Hedrick et al. 2003; Traxler, Kieser & Richard 1999)
- *Scomber japonicus*<sup>N</sup> (Pacific chub mackerel) (Hedrick et al. 2003)
- *Scophthalmus maximus*<sup>N,E</sup> (turbot) (Castric & de Kinkelin 1984; Schlotfeldt et al. 1991)
- *Solea senegalensis*<sup>N</sup> (Senegalese sole) (EFSA 2008)
- *Sprattus sprattus*<sup>N</sup> (sprat) (Mortensen et al. 1999)
- *Symphodus melops*<sup>N</sup> (corkwing wrasse) (Munro et al. 2015)
- *Thymallus thymallus*<sup>N</sup> (grayling) ((Wizigmann, Baath & Hoffmann 1980) cited in (Skall, Olesen & Møllergaard 2005b))
- *Thaleichthys pacificus*<sup>N</sup> (eulachon) (Hedrick et al. 2003)
- *Trachurus mediterraneus*<sup>N</sup> (Mediterranean horse mackerel) (Ogut & Altuntas 2014)
- *Trisopterus esmarkii*<sup>N</sup> (Norway pout) (Mortensen et al. 1999)
- *Uranoscopus scaber*<sup>N</sup> (Atlantic stargazer) (Ogut & Altuntas 2014).

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection (N=natural exposure; E=experimental exposure) with VHSV in accordance with chapter 1.5 of the WOA Code (WOAH 2022b) include:

- *Alosa pseudoharengus*<sup>N</sup> (alewife) (Stepien & Niner 2020)
- *Ammodytes personatus*<sup>N</sup> (sand eel) (Watanabe et al. 2002)
- *Anguilla anguilla*<sup>N</sup> (European eel) ((Castric et al. 1992) cited in (Skall, Olesen & Møllergaard 2005b))

- *Argentina sphyraena*<sup>N</sup> (lesser argentine) (Mortensen et al. 1999)
- *Belone belone*<sup>N</sup> (garfish) (Ogut & Altuntas 2014)
- *Catostomus commersonii* (white sucker) (WOAH 2022c)
- *Cottus pollux*<sup>E</sup> (Japanese fluvial sculpin) (Ito & Olesen 2013)
- *Dicentrarchus labrax*<sup>E</sup> (sea bass) (Castric & de Kinkelin 1984)
- *Enchelyopus cimbrius*<sup>N</sup> (fourbeard rockling) (Mortensen et al. 1999)
- *Esox lucius* × *Esox masquinongy* *Esox masquinongy* hybrids<sup>E</sup> (tiger muskellunge) (Getchell et al. 2013; Groocock et al. 2012)
- *Eutrigla gurnardus*<sup>N</sup> (gray gurnard) (Wallace et al. 2015)
- *Fundulus diaphanus*<sup>N</sup> (banded killifish) (Bain et al. 2010)
- *Gadiculus argenteus*<sup>N</sup> (silvery pout) (Sandlund et al. 2014)
- *Glyptocephalus stelleri*<sup>N</sup> (blackfin flounder) (Lee et al. 2007)
- *Hippoglossus hippoglossus*<sup>N</sup> (Atlantic halibut) (Bowden 2003)
- *Hoplobrotula armata*<sup>N</sup> (armoured cusk) (Lee et al. 2007)
- *Hypomesus pretiosus*<sup>N</sup> (surf smelt) (Hedrick et al. 2003)
- *Ictalurus punctatus*<sup>N</sup> (channel catfish) (Thompson et al. 2011)
- *Larimichthys polyactis*<sup>N</sup> (yellow croaker) (Lee et al. 2007)
- *Liparis tessellatus*<sup>N</sup> (cubed snailfish) (Lee et al. 2007)
- *Lota lota* (burbot)<sup>N</sup> (Thompson et al. 2011)
- *Melanogrammus aeglefinus*<sup>N</sup> (haddock) (Smail 2000)
- *Merluccius productus*<sup>N</sup> (north pacific hake) (Meyers, Short & Lipson 1999)
- *Moxostoma anisurum*<sup>N</sup> (silver redhorse) (Faisal et al. 2012)
- *Moxostoma macrolepidotum*<sup>N</sup> (shorthead redhorse) (Thompson et al. 2011)
- *Mugil cephalus*<sup>N</sup> (flathead grey mullet) (Lee et al. 2007)
- *Notemigonus crysoleucas*<sup>E</sup> (golden shiner) (Cornwell & Bellmund)
- *Oncorhynchus mykiss* × *Salvelinus alpinus* hybrids<sup>E</sup> (rainbow trout × arctic charr hybrids) (Dorson, Chevassus & Torhy 1991)
- *Oncorhynchus mykiss* × *Salvelinus namaycush* hybrids<sup>E</sup> (rainbow trout × lake trout hybrids) (Dorson, Chevassus & Torhy 1991)
- *Oncorhynchus mykiss* × *Salmo trutta* hybrids (rainbow trout × brown trout hybrids) (WOAH 2022c)
- *Oryzias latipes*<sup>E</sup> (Japanese rice fish) (Ito & Olesen 2013)
- *Oryzias dancena*<sup>E</sup> (marine medaka) (Kim, Oh & Oh 2013)



- *Pampus argenteus*<sup>N</sup> (silver pomfret) (Lee et al. 2007)
- *Percopsis omiscomaycus*<sup>N</sup> (trout perch) (Thompson et al. 2011)
- *Pomoxi annularis*<sup>N</sup> (white crappie) (Al-Hussinee et al. 2011)
- *Reinhardtius hippoglossoides*<sup>N</sup> (Greenland halibut) (Dopazo et al. 2002)
- *Rhinogobius species*<sup>E</sup> (Yoshinobori) (Ito & Olesen 2013)
- *Salvelinus alpinus*<sup>E</sup> (arctic charr) (Dorson, Chevassus & Torhy 1991)
- *Salvelinus fontinalis*<sup>E</sup> (brook trout) (Ogut & Altuntas 2011)
- *Scorpaena porcus*<sup>N</sup> (black scorpionfish) (Ogut & Altuntas 2014)
- *Scorpaena izensis*<sup>N</sup> (izu scorpionfish) (Lee et al. 2007)
- *Scyliorhinus torazame*<sup>N</sup> (claudy catshark) (Lee et al. 2007)
- *Semotilus corporalis* (fallfish) (WOAH 2022c)
- *Seriola dumerili* (greater amberjack) (WOAH 2022c)
- *Theragra chalcogramma*<sup>N</sup> (Alaska pollock) (Meyers, Short & Lipson 1999)
- *Trichiurus lepturus*<sup>N</sup> (largehead hairtail) (Lee et al. 2007)
- *Trisopterus minutus*<sup>N</sup> (poor cod) (King et al. 2001).

Other species that are reported as susceptible to infection (N=natural exposure; E=experimental exposure) with VHSV include:

- *Acanthopagrus schlegelii*<sup>E</sup> (blackhead seabream) (Isshiki, Nagano & Miyazaki 2003)
- *Clupea harengus membras*<sup>N</sup> (Baltic herring) (Gadd et al. 2011)
- *Epinephelus akaara*<sup>E</sup> (Hong Kong grouper) (Isshiki, Nagano & Miyazaki 2003).

VHSV-positive RT-PCR results or viral isolation in cell culture have been reported in a few species; however, no active infection was demonstrated (N= natural exposure; E= experimental exposure):

- *Acipenser baerii*<sup>E</sup> (Siberian sturgeon) (Ryu et al. 2018)
- *Anoplopoma fimbria*<sup>N</sup> (sablefish) (Traxler, Kieser & Richard 1999)
- *Chondrostoma polylepis*<sup>N</sup> (Iberian nase) (Lopez-Vazquez et al. 2003)
- *Sparus aurata*<sup>N</sup> (gilthead seabream) (Lopez-Vazquez et al. 2003).

VHSV may infect all life stages of fish except for eggs (Munro & Gregory 2010; WOA 2022c). 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 & S.E. 1999).

In experimental studies, clonal-cell lines established from *A. baerii* head-kidney tissues were susceptible to VHSV isolated from *Paralichthys olivaceus* (Ryu et al. 2018). The TCID<sub>50</sub> of VHSV on the HK7s4 clonal cell-line was determined and showed VHSV titre increased with time and at day 12 of culture was 10<sup>6.4</sup> TCID<sub>50</sub>/mL (Ryu et al. 2018). The same experiment was done on the HK10s8 cell line

and the VHSV titre on day 12 was  $10^{3.5}$  TCID<sub>50</sub>/mL (Ryu et al. 2018). There are no reports showing natural infection of sturgeon to VHSV or that sturgeon can be experimentally infected. WOAH does not list sturgeon as a susceptible species (WOAH 2022c). Despite this lack of evidence, sturgeon have been reported to be susceptible or vector species of VHSV (Communities 2008; Skall, Olesen & Møllergaard 2005b).

### 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

#### *Sturgeon*

No reports of the prevalence of VHSV in farmed or wild sturgeon were found.

#### *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 *C. harengus*, 11% (n=3038) in *S. sprattus*, 3.4% (n=1,272) in *L. limanda*, 8.4% (n=431) in *P. flesus*, 2.8% (n=531) in *P. platessa*, 14.3% (n=210) in *P. minutus*, 2.4% (n=252) in *Ammodytes* species and 0.2% (n=2039) in *G. morhua* (Skall, Olesen & Møllergaard 2005a). A 2009–2011 survey in the Black Sea for VHSV in marine fish found a maximum prevalence of 28% in *S. porcus* (n=3 pools), 28% in *U. scaber* (n=9 pools) and 22% in *T. mediterraneus* (n=5 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 *P. olivaceus* and 2% (n=52) of *A. personatus* (Watanabe et al. 2002). In Finland, VHSV was detected in 6.7% of *C. harengus membras* (n=758 pools where each pool contained 10 Baltic herring) collected during 2004–2006 (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 2005–2010 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 2022c).

### *Sturgeon*

No reports of mortalities in farmed or wild sturgeon due to VHSV were found.

### *Other fish*

Mortality often reaches 80–100% in *O. mykiss* fry and fingerlings and less in older fish, typically 25–75% depending on stressors present (Meyers & Winton 1995; Skall, Olesen & Møllergaard 2005b; Wolf 1988). Fish deaths due to a VHSV outbreak in farmed *O. mykiss* in the UK in 2006 peaked at approximately 2000 per day (Stone et al. 2008). In January–June 2021, VHSV outbreaks in Iran in *O. mykiss* resulted in 565,867 fish being slaughtered and an additional 101,383 deaths (WOAH 2022d). During the same period, a VHSV infection in *S. trutta* in the Czech Republic led to 14,500 mortalities (WOAH 2022d).

Mass mortality of *S. sagax* has frequently been reported along coastal British Columbia 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. 2013b). In the Bay of Quinte, Canada, VHSV infection of *A. grunniens* 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 *N. melanostomus* 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 2005–2008 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 (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-Hussiney et al. 2010; Eaton et al. 1991; Tuttle-Lau, Phillips & Gaikowski 2009).

Virus can be shed from infected fish via the urine (Neukirch & Glass 1984) and reproductive fluids ((Jorgensen 1970) cited in (Meyers & Winton 1995)). The shedding rates of VHSV from experimentally infected *C. pallasii* (by immersion) were estimated at a maximum of  $1.8\text{--}5.0 \times 10^8$  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 *E. masquinongy* (by immersion) shed upwards of  $1.36 \times 10^5$  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 & Møllergaard 2005b). For example, *O. mykiss* 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 *C. pallasii* following infection (Hershberger et al. 2010b). In clinically healthy *O. mykiss*, VHSV persisted for up to 14 weeks at 5°C (Jørgensen 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 & S.E. 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; WOA 2022c). For example, several VHSV infections among *C. pallasii* and *S. sagax* 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).  
Other

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 *Graptemys 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).

VHSV is considered to have originated in sea water and in marine fish species and then moved to the fresh water 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 *O. mykiss* was detected in 3 neighbouring sea sites within 3–4 months (Dale et al. 2009). At salmon 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 salmon (Garver et al. 2013b). Transport water may also contribute to spread of VHSV as it has been shown that water containing healthy but infected wild *C. pallasii* that were being transferred to a laboratory contained VHSV at concentrations of  $1.6 \times 10^2$ – $1.7 \times 10^3$  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 fish health oversight, 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

Bath challenge experiments using a UK VHSV isolate at 10 TCID<sub>50</sub>/mL for 4 hours could induce mortality in *O. mykiss* (weight 4.6–15.8 g) whereas a concentration of 10<sup>3</sup> TCID<sub>50</sub>/mL was required to cause mortality in *S. trutta* (mean weight 5 g) (Dixon, Joiner & Way 2007). Evensen et al (1994) bath challenged *O. mykiss* (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<sup>2</sup> TCID<sub>50</sub>/mL (Evensen et al. 1994). Bath exposure of *O. mykiss* (weight 20–30 g) with 3.5 × 10<sup>3</sup> TCID<sub>50</sub>/mL for 2 hours led to morbidity and mortality (Jonstrup et al. 2013). Juvenile *O. mykiss* (≤2 g) immersed for 1 hour in 10<sup>5</sup> PFU/mL showed mortality but none was seen with a dose of 10<sup>3</sup> PFU/mL (Meyers & Winton 1995). *O. mykiss* fry (mean weight 4.4 g) fed infected viscera (1.41 × 10<sup>4</sup> TCID<sub>50</sub>/mL) or brain and gill (4.45 × 10<sup>3</sup> TCID<sub>50</sub>/mL) homogenates resulted in 100% mortality after 7–11 dpi (Oidtmann et al. 2011a).

*Coregonus* fish (3 cm length) infected by immersion for 1 hour in 10<sup>4</sup> TCID<sub>50</sub>/mL at 11–12°C resulted in morbidity and mortality (Ahne & Thomsen 1985). Bath exposure of *S. maximus* (mean weight 8 g) for 3 hours to 8 × 10<sup>4</sup> TCID<sub>50</sub>/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 *C. pallasii* (mean length 100 mm; 1+ year old) to concentrations of VHSV at 10<sup>1</sup> PFU/mL for 24 hours or 10<sup>2</sup>–10<sup>3</sup> PFU/mL for 1 hour (Hershberger et al. 2010a; Kocan et al. 1997). *C. pallasii* (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 (LD<sub>50</sub>) of genotype IVb for juvenile *E. masquinongy* by intraperitoneal injection (weight 17.5 g) was 2.2 PFU and for immersion challenge (weight 0.7 g) was 1.7 × 10<sup>4</sup> PFU/mL (Kim & Faisal 2010).

## 18.2.2 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

Seawater *O. mykiss* that were either injected with or immersed in VHSV recorded virus titres of  $8 \times 10^6$ – $1 \times 10^8$  PFU/g and  $6 \times 10^5$ – $3 \times 10^8$  PFU/g pooled kidney and spleen tissue, respectively (Castric & de Kinkelin 1980). The results were similar for infected freshwater *O. mykiss*, ranging between  $4 \times 10^6$ – $2 \times 10^7$  PFU/g of tissue for injected fish and between  $2 \times 10^7$ – $2 \times 10^8$  PFU/g for bath exposed fish (Castric & de Kinkelin 1980). VHSV titres from *O. mykiss* infected by immersion challenge were  $2.11 \times 10^7$  TCID<sub>50</sub>/g internal organs (pooled kidney, spleen and liver),  $6.67 \times 10^6$  TCID<sub>50</sub>/g brain and gill and  $2.81 \times 10^6$  TCID<sub>50</sub>/g muscle tissue (Oidtmann et al. 2011a). In muscle and gill/brain tissue from infected market-size *O. mykiss* the VHSV titres were  $10^{1.0}$ – $10^{6.8}$  TCID<sub>50</sub>/g before onset of clinical signs,  $10^{0.5}$ – $10^{5.8}$  TCID<sub>50</sub>/g in clinically affected fish,  $10^{2.3}$ – $10^{6.6}$  TCID<sub>50</sub>/g in dead fish and  $10^{0.5}$ – $10^{6.7}$  TCID<sub>50</sub>/g in clinically healthy fish still alive 6 weeks after challenge with VHSV (Oidtmann et al. 2011b). Titres in bath infected *O. mykiss* ranged from  $2.7 \times 10^2$  to  $>1 \times 10^6$  TCID<sub>50</sub>/mL (Jonstrup et al. 2013). VHSV replication in excised skin of *O. mykiss* 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 *S. salar* ranged from  $4 \times 10^5$ – $1 \times 10^7$  PFU/g kidney tissue (Lovy et al. 2013). In surviving fish 10 weeks later, the titres were higher than  $1 \times 10^6$  PFU/g kidney tissue (Lovy et al. 2013).

Viscera from infected wild *C. pallasii* collected in Washington, USA had VHSV titres ranging from  $2 \times 10^3$ – $9 \times 10^4$  PFU/mL (Meyers & Winton 1995). Wild *C. pallasii* from Hoonah Harbour, Alaska had titres of VHSV ranging from  $3.16 \times 10^4$ – $5.62 \times 10^7$  TCID<sub>50</sub>/mL (Meyers & Winton 1995). Healthy but infected wild *C. pallasii* and *A. hexapterus* 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) *C. pallasii* (age 1+ year) resulted in VHSV titres of  $1.2 \times 10^3$ – $3.3 \times 10^7$  PFU/g of pooled kidney and spleen and  $7.1 \times 10^3$ – $2.2 \times 10^7$  PFU/g of brain in dead fish (Hershberger et al. 2010b). The VHSV titre in pooled kidney and spleen tissue from dead *C. pallasii* experimentally infected (by immersion) was  $5.7 \times 10^5$  PFU/g of and  $1.2 \times 10^4$  PFU/g in survivors (Hershberger et al. 2010a). VHSV titres from infected *S. sagax* ranged from  $1.4 \times 10^2$ – $1.6 \times 10^6$  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<sub>50</sub>/g (Ahne & Thomsen 1985).

## 18.2.3 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 2022c). 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 & Møllergaard 2005b; WOAH 2022c). The kidney, liver, spleen and

haematopoietic tissue typically show extensive multifocal necrosis and degeneration (WOAH 2022c). 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; WOA 2022c). 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 WOA *Manual of diagnostic tests for aquatic animals* (WOAH 2022c) 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 2022b).

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 2022c).

### 18.2.4 Treatment

There are currently no treatments for VHSV (WOAH 2022c).

### 18.2.5 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 *O. mykiss* 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 are control measures 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).

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. 2005b; WOA 2022c). It was estimated that 1 out of 100 disinfected *O. mykiss* 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 2022c). 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 *O. mykiss* (Chevassus & Dorson 1990; Dorson et al. 1995; Henryon et al. 2002; Verrier et al. 2013); however, no resistant *O. mykiss* strains are commercially available (WOAH 2022c).

### 18.2.6 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 & Møllergaard 2005b)). In 2000, the economic loss of VHSV outbreaks on 2 Danish fish farms producing approximately 165 tonnes *O. mykiss* was estimated to be above €211,000 with an associated total mortality of 50% ((Nylin & Olesen 2001) cited in (Skall, Olesen & Møllergaard 2005b)). VHSV outbreaks occurring in wild populations may also contribute to population declines. For example, the isolation of VHSV from *C. pallasii* 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).

### 18.2.7 Current biosecurity measures

There are no biosecurity measures for live sturgeon or their reproductive material as import is not permitted.

There are biosecurity measures to manage the risk of VHSV in imported bony fish for aquaculture or bait use (see [Appendix D](#)).

## 18.3 Risk assessment

Based on [chapter 4](#) and the technical information about VHSV presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of VHSV meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 18.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that VHSV is present in all source countries.



- There are no reports of VHSV infecting live sturgeon either by natural or experimental exposure. However, as VHSV were shown to infect sturgeon cell lines (Ryu et al. 2018), it will be assumed that VHSV can infect live sturgeon.
- VHSV would be likely to infect sturgeon life stages exported to Australia as VHSV infects fish of all life stages.
- Prevalence of VHSV in farmed salmonids can reach 100% and in wild populations can be up to 28%.
- There are no reports of VHSV associated with sturgeon reproductive material. Since it has been detected on other fish eggs and decontamination of eggs is commonly used to control VHSV infections it will be assumed that VHSV can associate with sturgeon reproductive material.
- VHSV can survive in freshwater and seawater for extended periods, particularly at low temperatures.
- The viral load of VHSV in infected imported live sturgeon or their reproductive material would likely be sufficient to cause infection in susceptible species.
- It is unknown if infected sturgeon would show clinical signs. However, if present, inspection may detect sturgeon showing clinical signs of infection with VHSV and remove them before export. Sturgeon showing mild or no clinical signs would be unlikely to be detected.
- Sturgeon reproductive material infected or contaminated with VHSV are unlikely to be detected during inspection because there would be no clinical signs.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the annual likelihood of entry of VHSV was estimated to be:

- Imported live sturgeon—**Low**.
- Imported sturgeon reproductive material—**Low**.

### 18.3.2 Exposure assessment

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

- VHSV can be transmitted horizontally via either by direct contact with diseased fish or indirect contact via contaminated water and equipment.
- VHSV which enters the environment would be capable of surviving for a period.
- VHSV would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- Species susceptible to VHSV infection are present in Australia include salmonids, *Anguilla* species, *Clupea* species, *H. armata*, *M. cephalus*, *S. dumerili*, *S. sagax*, and *Scomber* species.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are susceptible to VHSV infection.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is within the range that some VHSV genotypes cause infection.

- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infected sturgeon will be certain to be exposed to viable VHSV.
- Introduction into the wild may occur by direct release of imported live sturgeon or its associated wastes from the aquaculture facility into natural waters. This would be a direct pathway to wild susceptible species if a farm has not implemented standards of biosecurity for fish escapes and waste management that would exclude VHSV from discharges.
- Wild susceptible species would be less abundant than susceptible species in aquaculture facilities. Despite this, wild susceptible species would be expected to be exposed to VHSV released into natural waters due to the ability of the virus to survive independent of the host and susceptible species being present in Australian waters.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to VHSV in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

The partial likelihood of exposure to each exposure group to VHSV in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**Moderate**
- Wild susceptible species—**Low**.

### 18.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to VHSV in **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

The partial annual likelihood of entry and exposure of each exposure group to VHSV in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Very low**.

### 18.3.4 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 via either by direct contact with diseased fish or indirect contact via contaminated water and equipment. Transmission between broodstock and progeny occurs.
- 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.
- VHSV can infect salmonids, *Anguilla* species, *Clupea* species, *H. armata*, *M. cephalus*, *S. dumerili*, *S. sagax*, and *Scomber* species present in Australia.
- Aquaculture species most likely to be polycultured with imported sturgeon such as salmonids are susceptible to VHSV infection.
- Live sturgeon or sturgeon reproductive material could be moved to other aquaculture facilities in Australia. Species polycultured with VHSV-infected sturgeon or sharing the same water, could also be moved to other facilities. It is expected that VHSV would establish in these facilities if present in the animals or reproductive material being translocated.
- Each state and territory have translocation protocols for aquaculture animals, which typically include consideration of VHSV.
- If VHSV were to establish on a farm it could spread to neighbouring farms and wild populations through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of VHSV be suspected and response measures initiated immediately. However, VHSV is effectively transmitted through water and can persist in the environment and on farms which share a common water source or equipment with an infected population.
- The likelihood of VHSV spread from farms to wild populations or neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however VHSV could spread this way. VHSV could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.
- The use of infected farmed fish as feed or bait is another pathway for VHSV spread from farms to wild populations or neighbouring farms.
- If one or more index cases of VHSV were to occur in the wild, establishment and spread would be similar to on a farm because the wide range of susceptible animals increases the opportunities for transmission. However, because VHSV can survive in the environment, it could persist until susceptible hosts were to encounter it.
- The likelihood of VHSV in a wild population spreading to its natural geographic limits is greater than for other hazards with limited host ranges, for example, typical *Aeromonas salmonicida*, and would be more likely than for those hazards which cannot survive outside of a host for long periods. 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, especially in waters around aquaculture facilities, it may easily spread to farms through water intake due to VHSV being able to survive in the

environment and being transmissible through water. 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 4, the partial likelihood of establishment and spread of VHSV in each exposure group for the outbreak scenario was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

### Determining adverse impacts resulting from the outbreak scenario

The key points 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 AU\$1 billion in 2020–21 (Tuyman & Dylewski 2022).
- Sturgeon are assumed to be susceptible to VHSV. The establishment of a sturgeon industry in Australia 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*

- VHSV has a wide host range and there are reports of infection 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 WOA 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 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 control 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 WOA-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to VHSV to avoid the possible introduction of VHSV.
- If VHSV were to become established, Australia could use zoning to maintain access to international markets for live susceptible species, including sturgeon and, if required, 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.
- The impacts of VHSV 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 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 29 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 29 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)	Local	Unlikely to be discernible	A
	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**.

### 18.3.5 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:

- Farmed susceptible species—**High**.
- Wild susceptible species—**High**.

### 18.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of VHSV from **imported live sturgeon** 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

The partial annual risk of entry, establishment and spread of VHSV from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

### 18.3.7 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:

- Imported live sturgeon—**Moderate**.
- Imported sturgeon reproductive material—**Moderate**.

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

## 18.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for VHSV in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 18.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 30 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of VHSV but which on their own do not achieve Australia's ALOP for VHSV in imported live sturgeon or their reproductive material.

**Table 30 Biosecurity measures that on their own do not achieve Australia's ALOP for VHSV**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Disease-free stock	<b>Yes:</b> Determination of VHSV freedom would need to be to a standard consistent with that recommended by the World Organisation for Animal Health (WOAH), or equivalent.
2	Post-arrival quarantine (PAQ)	<b>Yes:</b> The stress of transport may induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
3	Post-arrival batch testing	<b>Yes:</b> Cell culture, real-time RT-PCR and amplicon sequencing of a conventional PCR product methods can detect VHSV (WOAH 2022b). Under this scenario, testing is conducted under departmental control and oversight.

### 18.4.2 Biosecurity measures that in combination achieve Australia's ALOP

A combination of biosecurity measures 1, 2 and 3 when applied to **imported live sturgeon** would reduce the likelihood of entry of VHSV from **low** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1, 2 and 3 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of VHSV from **low** to **negligible**.

This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP



# 19 *Yersinia ruckeri* (Hagerman strain)

## 19.1 Background

*Yersinia ruckeri* causes the bacterial disease yersiniosis in marine and freshwater fish species (Noga 2000; Vuillaume et al. 1987). The severity of disease is related to the strain of the bacterium and the host species involved. Strains of *Y. ruckeri* are classified based on biotype, serotype and outer-membrane protein type (Tobback et al. 2007). There are eight serotypes: O1 (subgroups O1a and O1b), O2 (subgroups O2a, O2b and O2c), O3 through to O8 (Gulla et al. 2018; Kumar et al. 2015; Ormsby & Davies 2021). Serotype O1a (Hagerman strain) is the most virulent serovar and the aetiological agent of enteric red mouth disease (ERM) (Romalde & Toranzo 1993; Ross, Rucker & Ewing 1966). *Y. ruckeri* (Hagerman strain) is the only strain 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 2022a) and has been retained as a hazard.

ERM is primarily a disease of salmonids and causes significant economic losses in the fish aquaculture industry. It is named after the subcutaneous haemorrhages it can cause at the corners of the mouth and in gums and tongue of affected fish (Kumar et al. 2015). ERM was first described in diseased *Oncorhynchus mykiss* (rainbow trout) in the Hagerman Valley in the United States of America (USA) in the 1950s (Ross, Rucker & Ewing 1966; Rucker 1966). It has since been reported in Asia, Europe, North and South America and South Africa (Tobback et al. 2007).

ERM is not listed as a disease notifiable to the World Organisation for Animal Health (WOAH) (OIE 2020) but is included on Australia's *National list of reportable diseases of aquatic animals* (AHC 2020). Australia has a long history of passive surveillance and a strong system in place to detect incursions. In Australia, *Y. ruckeri* serotype O1b (biotypes 1 and 2) and serotype O2 have been detected. The lineages of *Y. ruckeri* strains in Australia (and New Zealand) are distinct from those of the northern hemisphere, suggesting they are pre-existing ancient strains that have emerged and evolved with the introduction of susceptible hosts following European colonization (Barnes et al. 2016). *Y. ruckeri* serotype O1a (Hagerman strain) is considered exotic to Australia (Carson et al. 2019).

## 19.2 Technical information

For simplicity, *Y. ruckeri* (Hagerman strain) will be referred to as *Y. ruckeri* from here onwards.

### 19.2.1 Agent properties

*Y. ruckeri* is a gram-negative, rod-shaped bacterium that is a member of the family *Enterobacteriaceae* (Kumar et al. 2015; Noga 2000). There are two biotypes of *Y. ruckeri* (Tobback et al. 2007). Biotype 1 is positive for motility and secretes lipase and biotype 2 is negative when tested for motility and lipase secretion (Horne & Barnes 1999; Tobback et al. 2007).

*Y. ruckeri* can survive for several months outside the host in water, sediment or biofilms of fish tanks (Coquet et al. 2002; Furones, Rodgers & Munn 1993; Romalde et al. 1994b; Tobback et al. 2007). It can be cultured from frozen salmonid carcasses after more than 6 months storage (Anderson, Knowles & de Lisle 1994).

Water temperature is an important factor in the virulence of *Y. ruckeri*. The peak conditions for infection are 15–18°C and there is reduced morbidity and mortality below 10°C (Noga 2000).

*Y. ruckeri* can be inactivated by different disinfectants including potassium peroxymonosulfate, peracetic acid, hydrogen peroxide, ferric salt, chlorine, formic acid and povidone iodine (Bowker et al. 2016; Skall & Olesen 2011). *Y. ruckeri* can also be inactivated by heat treatment at 65°C for 5 minutes as well as ozone and UV treatment (Bowker et al. 2016; Skall & Olesen 2011).

## 19.2.2 Epidemiology

### Host range

Species which are reported to be susceptible to infection (N = natural exposure; E = experimental exposure) with *Y. ruckeri* includes but is not limited to:

- *Acipenser baerii*<sup>N</sup> (Siberian sturgeon) (Vuillaume et al. 1987)
- *Acipenser gueldenstaedtii*<sup>N</sup> (Russian sturgeon) (EURL for Fish and Crustacean Diseases 2021; Metcalf & Zajicek 2000)
- *Acipenser schrenckii*<sup>N</sup> (Amur sturgeon) (Shaowu et al. 2013)
- *Anguilla anguilla*<sup>N</sup> (eel) (Fuhrmann, Bohm & Schlotfeldt 1983)
- *Aristichthys nobilis*<sup>N</sup> (bighead carp) (Wrobel, Leo & Linke 2019)
- *Carassius auratus auratus*<sup>N</sup> (goldfish) (McArdle & Dooley-Martyn 1985)
- *Coregonus artedii*<sup>N</sup> (cisco) (Stevenson & Daly 1982)
- *Coregonus clupeaformis*<sup>N</sup> (whitefish) (Daly, Lindvik & Stevenson 1986)
- *Coregonus muksun*<sup>N</sup> (muksun) (Rintamaki, Valtonen & Frerichs 1986)
- *Coregonus peled*<sup>N</sup> (peled) (Rintamaki, Valtonen & Frerichs 1986)
- *Cyprinus carpio*<sup>N, E</sup> (carp) (Berc et al. 1999; Fuhrmann, Bohm & Schlotfeldt 1984)
- *Danio rerio*<sup>E</sup> (zebrafish) (Korbut et al. 2016)
- *Gadus morhua*<sup>N, E</sup> (Atlantic cod) (Gudmundsdottir et al. 2014)
- *Hypophthalmichthys molitrix*<sup>N</sup> (silver carp) (Wrobel, Leo & Linke 2019)
- *Lota lota*<sup>N</sup> (burbot) (Dwilow, Souter & Knight 1987)
- *Oncorhynchus clarkia*<sup>N</sup> (cutthroat trout) (Daly, Lindvik & Stevenson 1986)
- *Oncorhynchus kisutch*<sup>N</sup> (coho salmon) (Avendaño-Herrera et al. 2017)
- *Oncorhynchus mykiss*<sup>N, E</sup> (rainbow trout) (Rucker 1966)
- *Oncorhynchus nerka*<sup>N</sup> (sockeye salmon) (Ewing et al. 1978)
- *Oncorhynchus tshawytscha*<sup>N</sup> (chinook salmon) (Daly, Lindvik & Stevenson 1986)
- *Oreochromis niloticus*<sup>N</sup> (Nile tilapia) (Eissa et al. 2008)
- *Perca fluviatilis*<sup>N</sup> (European perch, redfin) (Valtonen, Rintamaki & Koskivaara 1992)
- *Pimephales promelas*<sup>N</sup> (fathead minnow) (Michel, Faivre & De Kinkelin 1986)

- *Pollachius virens*<sup>N</sup> (coalfish) (Willumsen 1989).
- *Rutilus rutilus*<sup>N</sup> (roach) (Valtonen, Rintamaki & Koskivaara 1992)
- *Salmo salar*<sup>N</sup> (Atlantic salmon) (Collins, Foster & Ross 1996)
- *Salmo trutta*<sup>N</sup> (brown trout) (Valtonen, Rintamaki & Koskivaara 1992)
- *Salvelinus alpinus*<sup>N</sup> (Arctic char) (Collins, Foster & Ross 1996)
- *Salvelinus fontinalis*<sup>N</sup> (brook trout) (Stevenson & Daly 1982)
- *Salvelinus malma*<sup>N</sup> (Dolly Varden trout) (Daly, Lindvik & Stevenson 1986)
- *Salvelinus namaycush*<sup>N</sup> (lake trout) (Daly, Lindvik & Stevenson 1986)
- *Scardinius erythrophthalmus hesperidicus*<sup>N</sup> (Rudd) (Popović, Hacmanjek & Teskeredžić 2001)
- *Scophthalmus maximus*<sup>N</sup> (turbot) (Michel, Faivre & De Kinkelin 1986)
- *Solea solea*<sup>N</sup> (Sole) (Michel, Faivre & De Kinkelin 1986).

*Y. ruckeri* has been isolated from numerous non-fish species, such as the European otter (Collins, Foster & Ross 1996). It has also been isolated from the intestine of sea gulls collected from around farms undergoing an outbreak of ERM (Willumsen 1989).

*Y. ruckeri* differs in virulence between host species and life stages (Haig et al. 2011). ERM can affect fish from all life stages, but fry and fingerling are most susceptible to acute disease. ERM appears as a more chronic condition in older fish (Kumar et al. 2015). Diseased sturgeon ranged from 15–30 g (Shaowu et al. 2013; Vuillaume et al. 1987).

### Geographical distribution

*Y. ruckeri* was first identified in the USA and has since been reported in Bulgaria, Canada, Chile, China, Croatia, Denmark, Finland, France, Germany, Hungary, Italy, Norway, Peru, Poland, Portugal, South Africa, Spain, Sweden, Republic of Türkiye, United Kingdom and Venezuela (Bravo & Kojagura 2004; Furones, Rodgers & Munn 1993; Kumar et al. 2015; Oraic et al. 2002; Rucker 1966; Shaowu et al. 2013; Tobback et al. 2007; Zorriehzahra, Adel & Delshad 2017).

### Prevalence

#### *Sturgeon*

A survey of bacterial infections in 6 sturgeon farms in northern Italy identified *Y. ruckeri* at a prevalence of 40% (n=5) in *A. gueldenstaedtii* in 2014 and 2015, 20% (n=5) in *A. baerii* in 2016 and 6.25% (n=16) in *H. huso* in 2017 (Santi et al. 2019). *Y. ruckeri* infection was also reported in *A. gueldenstaedtii* on a farm in Italy in 2020 (no numbers given) (EURL for Fish and Crustacean Diseases 2021).

#### *Salmonids*

In Germany in 2011–2012, 32.3% (n=431) of *O. mykiss* sampled from farms were positive for *Y. ruckeri* (Huang et al. 2015). There were 123 detections of *Y. ruckeri* reported to the Canadian Food Inspection Agency between 2013–2017 with most cases in *S. salar* (Wade 2019). Infection of farmed *O. mykiss* with *Y. ruckeri* was commonly reported throughout Europe during 2020 (EURL for Fish and Crustacean Diseases 2021). *Y. ruckeri* was reported at 19 sites in Norway in 2021 (Sommerset et al. 2022).

### *Other fish*

A prevalence of 66.6% (n=150) was reported in farmed *O. niloticus* experiencing an ERM outbreak in Egypt in 2007 (Eissa et al. 2008).

### **Mortalities**

Disease outbreaks typically start with sustained low-level mortalities which can progress to high levels (Shaowu et al. 2013; Tobback et al. 2007).

### *Sturgeon*

A cumulative mortality of 40% in *Y. ruckeri*-infected *A. baerii* farmed in France has been reported (Vuillaume et al. 1987). A *Y. ruckeri* outbreak that occurred in 2010 on a *A. schrenckii* farm in China caused mortalities (no numbers given) (Shaowu et al. 2013).

### *Salmonids*

Losses due to ERM in farmed salmonids can range from 30–70% of the stock (Busch 1978; Horne & Barnes 1999). There are limited recent publications about mortalities in wild or farmed populations of fish due to *Y. ruckeri*. Infections occurred in *O. mykiss* cage farms on the Black Sea coast of the Republic of Türkiye in 2002 and caused 3% mortality (Karatas, Candan & Demircan 2004). In 2008, some *S. salar* hatcheries in Chile suffered *Y. ruckeri* outbreaks with mortality up to 10% (Bastardo et al. 2011). Outbreaks in farmed *O. kisutch* in Chile in 2015 resulted in cumulative mortalities of 15% (Avendaño-Herrera et al. 2017).

### **Transmission**

*Y. ruckeri* infections can be transmitted by direct contact between fish or via shedding of the bacterium into the water column. The bacteria is typically shed in large numbers from infected or carrier fish through their faeces (Barnes 2011). *Y. ruckeri* can also form biofilms on surfaces and in sediments that are reported to be a source of recurrent infection in salmonid facilities (Coquet et al. 2002; Tobback et al. 2007).

Surviving fish can become carriers of the bacteria for extended periods. For example, *Y. ruckeri* could still be isolated from the faeces of *O. mykiss* 2 months after an ERM outbreak (Rodgers 1992). Experimentally infected *O. mykiss* that became carriers were capable of shedding bacteria for greater than 100 days (Busch & Lingg 1975). Unstressed carrier fish do not transmit the bacterium to other fish. However, under stressful conditions such as elevated water temperatures and poor water quality, carrier fish can release bacteria to cause infection in healthy fish (Hunter, Knittel & Fryer 1980). Serious epizootics in salmon have been traced to the introduction of carrier fish from infected sources (Cornick 1990).

True vertical transmission of *Y. ruckeri* has not been confirmed (Tobback et al. 2007). However, *Y. ruckeri* DNA has been detected in ovarian fluid, unfertilised eggs and iodophor-treated eyed sacs and sac fry of *O. tshawytscha* by PCR (Glenn et al. 2015). Further, the emergence of ERM in Venezuela is believed to have occurred through the importation of infected eggs (Furones, Rodgers & Munn 1993).

### **Infectious dose**

*A. schrenckii* (weight 40–60 g) injected with  $3 \times 10^7$  CFU *Y. ruckeri* displayed typical clinical signs of disease and mortality within 2–3 days post infection (dpi) (Shaowu et al. 2013). *A. schrenckii* (mean weight  $200 \pm 15$  g) challenged by intraperitoneal (IP) injection with  $1 \times 10^5$  CFU/g of fish body weight

resulted in no fish deaths during the 24 hours post-injection but several genes important to the immune response were up- or down-regulated in the spleen of infected animals (Li et al. 2017).

*O. mykiss* fry challenged with *Y. ruckeri* by immersion for 1 hour in 10L of water containing  $1 \times 10^8$  CFU/mL resulted in 33% mortality over the 21 days of the challenge (Ohtani et al. 2014). Mortality of 100% was observed in *O. mykiss* (mean weight 8 g) IP challenged with a non-motile strain of *Y. ruckeri* ( $1.3 \times 10^3$ – $1.3 \times 10^7$  CFU/mL) (Bastardo, Ravelo & Romalde 2012). In *O. mykiss* IP challenged with a motile *Y. ruckeri* isolate, 100% cumulative mortality was observed when concentrations between  $1.5 \times 10^4$ – $1.5 \times 10^7$  CFU/mL were used (Bastardo, Ravelo & Romalde 2012). Bath challenge of *O. mykiss* (mean weight 11 g) with  $1.7 \times 10^7$  CFU/mL of *Y. ruckeri* for 1 hour resulted in 41.6% mortality (Glenn, Taylor & Hanson 2011). *O. tshawytscha* (mean weight 15 g) bath exposed to  $2.6 \times 10^7$  CFU/mL of *Y. ruckeri* for 35–45 minutes suffered 66.1% mortality (Glenn, Taylor & Hanson 2011). Experimental challenge (by immersion) of *S. salar* fry (weight 0.4 g) with  $1.2$ – $4.8 \times 10^7$  CFU/mL and parr (weight 5–10 g) with  $1.19 \times 10^7$ – $1.3 \times 10^8$  CFU/mL *Y. ruckeri* for 4 hours induced mortality (Haig et al. 2011).

Mortality approached 90% in *C. carpio* IP injected with  $5 \times 10^5$  cells of *Y. ruckeri* (Berc et al. 1999).

### 19.2.3 Pathogenesis

#### Tissue tropism

It is thought that the key portal of entry for *Y. ruckeri* is either through the gill lamellae, which then spreads to the blood system and into the intestine and other parts of the host (Ohtani et al. 2014), or the nares, spreading up the olfactory nerve to the brain (Das & Salinas 2020; Ohtani et al. 2015). *Y. ruckeri* has been recovered from internal organs such as the kidney, intestine, spleen, brain, heart and liver (Ohtani et al. 2014; Rucker 1966; Shaowu et al. 2013). Ovarian fluid from spawning female adults, unfertilised eggs, eyed eggs and sac fry have also tested positive for *Y. ruckeri* DNA (Glenn et al. 2015).

#### Tissue titre

Following challenge of *O. mykiss* with various dilutions of a motile or non-motile isolate of *Y. ruckeri*, the amount of *Y. ruckeri* in the liver, kidney and spleen of surviving *O. mykiss* ranged from  $8.8 \times 10^2$ – $7.2 \times 10^5$  CFU/g (Bastardo, Ravelo & Romalde 2012). In the blood, loads of *Y. ruckeri* were  $6.2 \times 10^5$  CFU/mL (Bastardo, Ravelo & Romalde 2012). In comparison, the loads of *Y. ruckeri* ranged from  $1.3 \times 10^7$ – $9.8 \times 10^{10}$  CFU/g in the liver, kidney and spleen and  $7.5 \times 10^8$ – $9.5 \times 10^{10}$  CFU/mL in the blood of deceased *O. mykiss* following challenge (Bastardo, Ravelo & Romalde 2012). Juvenile *O. tshawytscha* naturally infected had  $10^{4.3}$  DNA copies/g kidney tissue (Glenn et al. 2015). *Y. ruckeri* was isolated from gills, gut, liver, spleen and kidney in the range of  $10^5$ – $10^8$  CFU/g tissue of *O. mykiss* that died after bath challenge and in the range of  $10^2$ – $10^3$  CFU/g tissue in fish that survived (Tobback et al. 2009). The mean number of *Y. ruckeri* in the kidneys of fish following bath exposure reached  $8.71 \times 10^7$  copies/g at 8 dpi for *O. mykiss* and  $2.77 \times 10^9$  copies/g at 6 dpi for *O. tshawytscha* (Glenn, Taylor & Hanson 2011). Glenn et al (2011) suggested that fish with *Y. ruckeri* loads lower than  $1 \times 10^7$  copies/g of kidney tissue are in a carrier state (Glenn, Taylor & Hanson 2011).

### 19.2.4 Diagnosis

#### Clinical signs

Signs of disease may include exophthalmia, darkening of the skin, haemorrhagic congestion at the base of the pectoral and pelvic fins, blood spots in the eye and subcutaneous haemorrhages in and

around the mouth and throat (Busch 1978; Kumar et al. 2015; Ohtani et al. 2014; Tobback et al. 2007). Changes in fish behaviour may be observed, including swimming near the surface, lethargy and loss of appetite. Clinical signs can develop within several days after a stressful event (Noga 2000). In some cases, infected fish show no clinical signs (Busch 1978; Kawula, Lelivelt & Orndorff 1996).

Incubation time is usually 5–7 days from initial exposure to commencement of mortality at 15°C. However, a population of fish with a prior history of disease will experience mortality in a reduced time of 3–5 days following a shedding event (Hunter, Knittel & Fryer 1980).

Most descriptions of the clinical signs of *Y. ruckeri* infection are reported for salmonids. However, the few reports related to sturgeon indicate similar clinical changes due to infection. Farmed *A. baerii* and *A. schrenckii* infected with *Y. ruckeri* were reported to have haemorrhages around the mouth, lower jaw, at the base of the rostrum and pectoral fins, the abdomen and around the vent (Shaowu et al. 2013; Vuillaume et al. 1987). In infected *A. baerii*, the belly was swollen and a bloody liquid exuded from the vent (Vuillaume et al. 1987).

### Pathology

Fish infected with *Y. ruckeri* generally display histological signs characteristic of septicaemia, with inflammation in most organs, especially kidney, spleen, liver, heart, gills and in areas with petechial haemorrhage. Petechial haemorrhages may occur on the surfaces of the liver, pancreas, pyloric caeca, swim bladder and in the lateral muscles. The spleen is often enlarged and can be almost black in colour, and the lower intestine can become reddened and filled with an opaque, yellowish fluid. Pathological changes in the gills include hyperaemia, oedema and desquamation of the epithelial cells in the secondary lamellae. Focal areas of necrosis may be seen in the spleen, kidney and liver. Degenerated renal tubules, glomerular nephritis and a marked increase in melano-macrophages may be observed in kidneys of affected fish (Barnes 2011; Busch 1978; Horne & Barnes 1999; Tobback et al. 2007; Tobback et al. 2009).

Affected sturgeon displayed petechiae in the liver, hindgut and swim bladder as well as haemorrhaging in the kidney, liver and spleen (Shaowu et al. 2013; Vuillaume et al. 1987).

### Testing

A diagnosis is usually based on clinical signs, isolation in culture of *Y. ruckeri* from systemic sites such as kidney or spleen and phenotypic profiling (Barnes 2011; Carson et al. 2019; Horne & Barnes 1999; Tobback et al. 2007). *Y. ruckeri* can also be detected using serological tests such as enzyme-linked immunosorbent assay, agglutination and immunofluorescence assay (Smith, Goldring & Dear 1987). Molecular detection methods include restriction fragment length polymorphism (Garcia et al. 1998), loop-mediated isothermal amplification (Saleh, Soliman & El-Matbouli 2008), PCR (Altinok, Grizzle & Liu 2001; Gibello et al. 1999) and real-time PCR (Bastardo, Ravelo & Romalde 2012).

## 19.2.5 Treatment

*Y. ruckeri* is generally sensitive to the range of antibiotics used routinely in aquaculture such as amoxicillin, oxolinic acid, oxytetracycline and florfenicol (Calvez et al. 2014; Inglis & Richards 1991; Michel, Kerouault & Martin 2003; Schmidt et al. 2000). However, repeated antibiotic treatments may be required and *Y. ruckeri* isolates can develop resistance (Alderman & Hastings 1998; Noga 2000). Infected farmed *A. baerii* have been treated with a combination of flumequine and oxolinic acid

(Vuillaume et al. 1987) and experimentally infected *A. baerii* were successfully treated with florfenicol (Wang et al. 2012).

There is evidence that probiotics can provide a protective effect against *Y. ruckeri* infections. For example, dietary supplementation with *Bacillus* species and *Aeromonas sobria* reduced *O. mykiss* mortalities due to *Y. ruckeri* to 0% and 6%, respectively, compared to 80% in the control (Brunt, Newaj-Fyzul & Austin 2007).

### 19.2.6 Control and prevention

Prevention of ERM outbreaks is achieved by avoiding the introduction of infected stock and ensuring that all imported eggs have been disinfected properly (Barnes 2011). Although, iodophor disinfectants are not always 100% effective against *Y. ruckeri* (Yamasaki et al. 2017). Other control measures include vaccination, good husbandry, good water quality, low stocking densities, minimisation of stress and the bacteria generally responds well to antibiotics (Barnes 2011). ERM was one of the first diseases for which an effective commercial fish vaccine was developed (Bowker et al. 2016; Kumar et al. 2015; Sommerset et al. 2005a). However, under field conditions the effectiveness of the vaccines may vary (Barnes 2011).

### 19.2.7 Impact of the disease

ERM is a serious infectious disease in the salmonid farming industry that causes large economic losses in many countries (Ahmed et al. 2014; Austin & Austin 1987; Glenn et al. 2015). It is considered one of the most significant diseases of freshwater trout aquaculture (Arias et al. 2007). Losses are due to direct mortalities as well as indirect costs due to stunted growth, delayed harvests, management procedures and treatments. In 1998, the British Trout Association estimated losses of £1.3–1.5 million (Barnes 2019). Recent reports on the economic impacts of ERM are not available.

Where ERM is present, endemic losses of 10–15% over a growth cycle are common, and individual outbreaks may result in much higher mortalities (Barnes 2011). However, the development of effective vaccines has reduced the impact of the disease substantially.

### 19.2.8 Current biosecurity measures

There are no biosecurity measures for live sturgeon or their reproductive material as import is not permitted.

There are biosecurity measures to manage the risk of *Y. ruckeri* in imported salmonid fish for human consumption (see [Appendix D](#)).

## 19.3 Risk assessment

Based on [chapter 4](#) and the technical information about *Y. ruckeri* presented in this chapter, a risk assessment was completed.

A summary of the risk assessment values for determining if the overall annual risk of *Y. ruckeri* meets Australia's appropriate level of protection (ALOP) are shown in [Appendix B](#) and [Appendix C](#).

### 19.3.1 Entry assessment

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

- This biosecurity import risk analysis (BIRA) is generic and therefore the entry assessment assumes that *Y. ruckeri* is present in all source countries.
- *Y. ruckeri* is primarily a disease of salmonids, but a wide range of non-salmonid species are susceptible to infection, including *Acipenser* species.
- *Y. ruckeri* is expected to infect sturgeon life stages that would be exported to Australia.
- There is only one report of *Y. ruckeri* prevalence in farmed sturgeon where it was detected in 6–40% of sampled fish from Italian farms (Santi et al. 2019). The prevalence of *Y. ruckeri* in wild sturgeon is unknown.
- Prevalence in farmed and wild non-sturgeon species can be up to 70%.
- There are no reports of *Y. ruckeri* associating with sturgeon reproductive material but disinfection of salmonid eggs to prevent ERM is a routine practice in hatcheries.
- *Y. ruckeri* can remain viable outside the host for extended periods.
- The bacterial load of *Y. ruckeri* in infected imported live sturgeon or their reproductive material is likely to be sufficient to cause infection in susceptible species.
- Inspection may detect sturgeon showing clinical signs of infection with *Y. ruckeri* and remove them before export. However, subclinically infected or carrier fish would not be identified through visual inspection.
- Sturgeon reproductive material infected or contaminated with *Y. ruckeri* are unlikely to be detected during inspection because there would be no clinical signs.

### Conclusion

Based on this information and using the qualitative likelihood descriptor in Table 4, the annual likelihood of entry of *Y. ruckeri* was estimated to be:

- Imported live sturgeon—**Moderate**.
- Imported sturgeon reproductive material—**Moderate**.

### 19.3.2 Exposure assessment

The key points considered relevant when conducting the exposure assessment for *Y. ruckeri* were that:

- *Y. ruckeri* can be transmitted horizontally via water and contact between fish. It may persist for an extended period in the environment (more than 3 months) (Romalde et al. 1994a).
- *Y. ruckeri* would be expected to be present in sufficient loads in imported live sturgeon or their reproductive material to cause infection in susceptible species if exposed.
- *Y. ruckeri* can infect a moderate range of species present in Australia, including salmonids, *Anguilla* species, *C. auratus*, *C. carpio*, *P. fluviatilis* and *R. rutilus*.
- Aquaculture species most likely to be polycultured with imported sturgeon such as trout are susceptible to *Y. ruckeri*.
- Sturgeon is typically cultured between 15–20°C (Castellano et al. 2017; Mohler 2003), which is in the range of peak severity (15–18°C) of ERM (Noga 2000).



- Because of the culture conditions in aquaculture facilities (e.g. high stocking densities), any farmed susceptible species grown with, or sharing the same water as infected sturgeon will be certain to be exposed to viable *Y. ruckeri*.
- Farmed susceptible species are generally stocked at relatively high densities and are not usually subject to competition from non-aquaculture species. For this reason, it is almost certain that any imported live sturgeon introduced to farmed susceptible species would make contact with this exposure group.
- Introduction into the wild may occur by direct release of imported live sturgeon or its associated wastes from the aquaculture facility into natural waters. Whilst wild susceptible species would be less abundant than susceptible species in aquaculture facilities, this would be a direct exposure pathway if a farm has not implemented standards of biosecurity for fish escapes and waste management that would exclude *Y. ruckeri* from discharges.

### Conclusion

Based on this information and using the qualitative likelihood descriptors in Table 4, the partial likelihood of exposure of each exposure group to *Y. ruckeri* in **imported live sturgeon** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

The partial likelihood of exposure to each exposure group for *Y. ruckeri* in **imported sturgeon reproductive material** was estimated to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Low**.

### 19.3.3 Determination of the partial annual likelihood of entry and exposure

The partial annual likelihood of entry and exposure of each exposure group to *Y. ruckeri* in **imported live sturgeon** 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:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Low**.

The partial annual likelihood of entry and exposure of each exposure group to *Y. ruckeri* in **imported sturgeon reproductive material** was similarly determined and found to be:

- Farmed susceptible species—**Low**.
- Wild susceptible species—**Low**.

### 19.3.4 Consequence assessment

#### Partial likelihood of establishment and spread

The key points considered relevant when determining the partial likelihood of establishment and spread of *Y. ruckeri* were that:

- *Y. ruckeri* can be transmitted horizontally via water and contact between fish. It may persist for an extended period in the environment (more than 3 months) (Romalde et al. 1994a). Transmission from broodstock to progeny may occur (Glennon et al. 2018).
- Fish that survive *Y. ruckeri* infection can remain carriers and become sources of the bacteria.
- *Y. ruckeri* can remain infectious in the environment for an extended time, particularly in biofilms and sediment.
- It is expected that susceptible species in contact with infected fish would receive an infectious dose.
- *Y. ruckeri* can infect a wide range of species present in Australia including salmonids, *Anguilla* species, *C. auratus*, *C. carpio*, *P. fluviatilis* and *R. rutilus*.
- Outbreaks of *Y. ruckeri* typically occur at water temperatures of 15– 18°C. Subclinical infections may occur in cooler waters (<10°C).
- *Y. ruckeri* can be treated by antibiotics although antibiotic-resistance strains can occur. Trimethoprim (Dihydrofolate Inhibitor) and Oxytetracycline (Tetracycline) are used in Tasmania's salmonid aquaculture industry (Aquaculture 2021).
- There are vaccines to control for *Y. ruckeri* (Hagerman strain) but they are not used in Tasmania's salmonid aquaculture industry (Tasmanian Government 2022).
- *Y. ruckeri* establishment, following a given quantity of the bacteria entering the environment of an exposure group, is likely for farmed susceptible species. This is due to the stressors associated with intensive aquaculture. For example, the higher density of susceptible animals and the culture conditions.
- Live sturgeon or their reproductive material could be moved to other facilities in Australia for further grow out. It is assumed that species polycultured with *Y. ruckeri*-infected sturgeon or in the same water, could also be moved for further grow out in another facility in Australia. It is expected that *Y. ruckeri* would establish in these facilities if present in the animals or reproductive material being translocated.
- Each state and territory have translocation protocols for aquaculture animals that typically includes consideration of *Y. ruckeri*.
- If *Y. ruckeri* were to establish on a farm it could spread to neighbouring farms and wild populations through wastewater. This spread would be moderated by dilution effects and implementation of biosecurity measures should an incursion of *Y. ruckeri* be suspected and response measures initiated immediately. However, *Y. ruckeri* is effectively transmitted through water and can persist in the environment and farms which share a common water source or equipment with an infected population may be exposed to *Y. ruckeri*.
- The likelihood of *Y. ruckeri* spread from farms to wild populations or neighbouring farms via escaped fish would be reduced due to the systems in place on farms to prevent discharge of live animals, however *Y. ruckeri* could spread this way. *Y. ruckeri* could also be spread from farms to wild populations via birds scavenging infected dead or moribund fish and dropping them into unaffected waters.

- Spread of *Y. ruckeri* from farmed to wild susceptible species may occur through the movement of infected polyculture species (e.g. rainbow trout, brown trout and Chinook salmon) into natural waters to replenish depleted populations. *Y. ruckeri* could be effectively transferred this way because fish may not show clinical signs before transfer.
- If one or more index cases of *Y. ruckeri* were to occur in the wild, establishment and spread would be similar as to on a farm because the densities of susceptible animals increases the opportunities for transmission. Because *Y. ruckeri* can survive in the environment, it could also persist until susceptible hosts were to encounter it.
- The likelihood of *Y. ruckeri* in a wild population spreading to its natural geographic limits is greater than for other hazards with limited host ranges, for example, typical *Aeromonas salmonicida*, and would be more likely than for those hazards which cannot survive outside of a host for long periods. The ability of fish to be subclinically infected with *Y. ruckeri* and to remain carriers after surviving an infection also aids its spread.
- If *Y. ruckeri* were to establish in the wild, especially in waters around aquaculture facilities, it may easily spread to farms through water intake due to *Y. ruckeri* being able to survive in the environment and being transmissible through water. In the absence of effective biosecurity measures, wild infected fish may be transferred into the farms through the inlet water channels.
- Once established, *Y. ruckeri* can spread rapidly over broad regions. *Y. ruckeri* has spread throughout Europe since its first introduction from the USA in the late 1970s (Wheeler et al. 2009).

## Conclusion

Based on this information and using the descriptors in Table 4, the partial likelihood of establishment and spread of *Y. ruckeri* in each exposure group for the outbreak scenario (refer section [Identification of the outbreak scenario](#)) was estimated to be:

- Farmed susceptible species—**Moderate.**
- Wild susceptible species—**Moderate.**

## Determining adverse impacts resulting from the outbreak scenario

The key points considered relevant when determining the adverse impacts resulting from establishment and spread of *Y. ruckeri* were that:

### *Direct effects*

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

- *Y. ruckeri* is primarily a disease of farmed salmonids. An outbreak of *Y. ruckeri* in farmed salmonids would likely lead to significant mortalities, and if established, on-going management would impact production. Production was valued at approximately AU\$1 billion in 2020–21 (Tuytman & Dylewski 2022).
- Mortality has also been associated with infection in farmed sturgeon. Production losses in sturgeon aquaculture would be significant considering sturgeon require >3 years to reach sexual maturity.
- *Y. ruckeri* is not likely to impact wild fisheries in Australia. There are no reports of *Y. ruckeri* mortalities in wild fish.

- Based on the impacts of *Y. ruckeri* in salmonid farming, its 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*

- *Y. ruckeri* can infect a moderate range of species present in Australia.
- There are no reports about serious effects of *Y. ruckeri* on wild fish populations. It is assumed susceptible wild fish (including salmonids) will not die from infection with *Y. ruckeri* but may become subclinical carriers of the disease.
- The direct impact of *Y. ruckeri* 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*

- ERM is not listed as a notifiable disease by WOAHA but is included on Australia's *National list of reportable diseases of aquatic animals*. State and territory governments are required to report on the presence of *Y. ruckeri* (Hagerman strain).
- If *Y. ruckeri* was confirmed in the environment, eradication would be near impossible as the agent is able to persist in biofilms and sediments.
- If infected animals were confined to a land-based farm, then an attempt at eradication is more likely. The cost of an eradication attempt in affected salmonid farms would be significant for the industry.
- If a movement restriction area were put in place for an outbreak of *Y. ruckeri*, there would be ongoing costs associated with the surveillance, monitoring and implementation of the area.
- 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 eradication was unsuccessful, preventative vaccination programs may be developed to control the spread of *Y. ruckeri* or manage the production of a susceptible species. Tasmania already vaccinates for *Y. ruckeri* serotype O1b.
- Eradication and control of *Y. ruckeri* is expected to cause a minor impact 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*

- 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.
- Movement control orders, if put in place, would have indirect impacts on other industries such as seafood suppliers due to the moderate host range of *Y. ruckeri*.
- *Y. ruckeri*-infected fish may show clinical signs which would affect their marketability. It is expected that *Y. ruckeri* would affect caviar production.

- *Y. ruckeri* 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 *Y. ruckeri* (Hagerman strain) is not a WOA-listed disease. Importing countries may have import requirements or have closed borders to the import of live, fresh or frozen species susceptible to *Y. ruckeri* to avoid the possible introduction of *Y. ruckeri*.
- If *Y. ruckeri* were to become established, Australia could use zoning to maintain or gain access to international markets for live fish and, if required, non-viable product.
- The impacts of *Y. ruckeri* 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*

- *Y. ruckeri* has a moderate host range but is not considered to cause significant mortality in wild susceptible fish.
- There are no species listed as endangered in Australia that are related to species known to be susceptible to *Y. ruckeri*.
- The impact of *Y. ruckeri* establishment and spread on the biodiversity of the environment 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*

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

Table 31 shows the individual impact scores for each criteria (determined using Figure 5) for establishment and spread of *Y. ruckeri*. 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 31 Overall impact of establishment and spread of *Y. ruckeri* 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)	Local	Unlikely to be discernible	A
	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 *Y. ruckeri* was estimated to be **high**.

### 19.3.5 Determination of likely consequences of the outbreak scenario

The likely consequences of the outbreak scenario for *Y. ruckeri* 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:

- Farmed susceptible species—**High**.
- Wild susceptible species—**High**.

### 19.3.6 Determination of the partial annual risk

The partial annual risk of entry, establishment and spread of *Y. ruckeri* from **imported live sturgeon** 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:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

The partial annual risk of entry, establishment and spread of *Y. ruckeri* from **imported sturgeon reproductive material** for each exposure group was similarly determined and found to be:

- Farmed susceptible species—**Moderate**.
- Wild susceptible species—**Moderate**.

### 19.3.7 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 *Y. ruckeri* (Hagerman strain) was found to be:

- Imported live sturgeon—**High**.
- Imported sturgeon reproductive material—**High**.

Therefore, as the overall annual risk does not achieve Australia's ALOP for *Y. ruckeri* (Hagerman strain), specific biosecurity measures are considered necessary for this hazard.

## 19.4 Biosecurity measures

Details of the biosecurity measures (and risk assessment values) considered to manage the risk for *Y. ruckeri* (Hagerman strain) in imported live sturgeon or their reproductive material to a level that meets Australia's ALOP are presented here and summarised in [Appendix B](#) and [Appendix C](#).

### 19.4.1 Biosecurity measures that on their own do not achieve Australia's ALOP

Table 32 summarises the biosecurity measures that were considered to reduce the **entry likelihood** of *Y. ruckeri* (Hagerman strain) but which on their own do not achieve Australia's ALOP for *Y. ruckeri* (Hagerman strain) in imported live sturgeon or their reproductive material.

**Table 32 Biosecurity measures that on their own do not achieve Australia's ALOP for *Y. ruckeri* (Hagerman strain)**

Number	Biosecurity measure	Reduces entry likelihood? (Yes/No: reason)
1	Sourcing from disease-free stocks	<b>Yes:</b> Determination of <i>Y. ruckeri</i> (Hagerman strain) freedom would be to a standard consistent with that recommended for World Organisation for Animal Health (WOAH) listed diseases, or equivalent.
2	Post-arrival quarantine (PAQ)	<b>Yes:</b> The stress of transport can induce clinical infection in live sturgeon that may be detected during the PAQ period. Producing sturgeon progeny from reproductive material and culturing for a period under conducive conditions for a clinical infection to appear may similarly detect infected sturgeon. However, subclinical infections may not be induced and detected in the PAQ period.
3	Post-arrival batch testing	<b>Yes:</b> Bacterial culture in combination with phenotypic profiling (Barnes 2011; Carson et al. 2019; Horne & Barnes 1999; Tobback et al. 2007), PCR (Altinok, Grizzle & Liu 2001; Gibello et al. 1999) and real-time PCR (Bastardo, Ravelo & Romalde 2012) can detect <i>Y. ruckeri</i> (Hagerman strain). Under this scenario, testing is conducted under departmental control and oversight.

### 19.4.2 Biosecurity measures that in combination achieve Australia's ALOP

A combination of biosecurity measures 1, 2 and 3 when applied to **imported live sturgeon** would reduce the likelihood of entry of *Y. ruckeri* (Hagerman strain) from **moderate** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

A combination of biosecurity measures 1, 2 and 3 when applied to **imported sturgeon reproductive material** would reduce the likelihood of entry of *Y. ruckeri* (Hagerman strain) from **moderate** to **negligible**.

- This would reduce the overall restricted risk to **negligible**, thereby achieving Australia's ALOP.

## 20 Proposed biosecurity measures for the importation of live sturgeon and their reproductive material for aquaculture

Several biosecurity measures are proposed to manage the risk of hazards within or on live sturgeon and their reproductive material imported to Australia for aquaculture.

Those seeking alternative biosecurity measures should provide a submission to the department for consideration. Such proposals should include supporting scientific data that explains the extent to which the alternative measures would achieve Australia's ALOP. Biosecurity measures which require case-by-case assessment were not considered in detail for each hazard as part of this biosecurity import risk analysis (BIRA).

### 20.1 General biosecurity measures

There are general biosecurity measures that will apply to live sturgeon and/or their reproductive material including:

- The sturgeon must be resident in an aquaculture premises under the supervision of the competent authority in the exporting country since birth or for at least 6 months immediately before export, unless otherwise approved by the department.
- For sturgeon reproductive material, the donor sturgeon must be resident in an aquaculture premises under the supervision of the competent authority in the exporting country since birth or for at least 6 months immediately before collection, unless otherwise approved by the department.
- The sturgeon must only be cultured with sturgeon and not with other fish or amphibians.
- Fertilised eggs must undergo a standard disinfection procedure prior to export and on arrival in Australia.
- The premises must provide separation from other fish populations, be under supervision of an aquatic health professional and have a documented health monitoring program that would be effective in monitoring for disease agents and the hazards identified in this BIRA (e.g. post-mortem investigation of deceased fish, disease testing programs, disease surveillance programs).
  - A health monitoring program is the regular monitoring, ongoing surveillance, and health oversight to ensure that the health status of the sturgeon and the aquaculture facility is known and monitored over time. This underpins official certification.
  - The aquatic health professional must have knowledge of the sturgeons' health status and the general health status of the aquatic facility that allows a certifying official to sign off on these records.



- Separation of the sturgeon must achieve a sufficient distance or other barriers to other fish populations to maintain a distinct animal health status with regards to the hazards in this BIRA.

## 20.2 Proposed biosecurity measures for the importation of live sturgeon for aquaculture

### 20.2.1 Documentation

Importers must obtain a permit from the Department of Agriculture, Fisheries and Forestry (the department) to import live sturgeon into Australia for aquaculture purposes before the fish are imported.

Importers will also require a Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permit and will need to submit an application through the [Department of Climate Change, Energy, the Environment and Water](#).

Each consignment of live sturgeon must travel with an original international health certificate that the department has agreed to, conforms to Chapter 5.11. of the World Organisation for Animal Health *Aquatic Animal Health Code* (WOAH Code) and is signed by a member of the personnel of the Competent Authority (CA) or a certifying official approved by the country of export.

A certifying official means a person authorised by the CA of the country of export to sign health certificates for aquatic animals in accordance with the Certification Procedures of Chapter 5.2 of the WOAH Code.

The health certificate must:

- be written in English and a language understood by the certifying official of the country of export
- meet the requirements of [section 20.2.3 Certification](#) and state that all the pre-export quarantine and biosecurity measure requirements have been met
- provide identification for each consignment of live sturgeon including species, sex, age and number of animals
- include the name and address of the aquaculture facility of origin
- include the name and address of the exporter and importer and identify the import permit against which it was issued.

The certifying official must:

- provide a health certificate that is specific to the consignment of live sturgeon that it covers
- sign, date and stamp (with the stamp of the CA) each page of the health certificate and any attached documents that form part of the extended health certification
- sign and stamp any manual deletions to the health certificate
- record their name, signature and official contact details on the health certificate.

## 20.2.2 Pre-export quarantine requirements

### Location

The pre-export quarantine (PEQ) facility must be under the supervision of the CA and subject to a health monitoring program that can address Australia's biosecurity requirements.

### Facilities

Requirements that may apply to the PEQ facility include:

- The PEQ facility must meet the country and premises requirements specified in [section 20.2.3 Certification](#).
- The PEQ facility must be surrounded by physical and procedural barriers that provide sufficient security to isolate the consignment of sturgeon in PEQ from all other fish except those that meet all the conditions in these biosecurity measures.
  - This is to ensure the quarantined sturgeon are protected from disease transmission, which includes direct contact, direct and indirect water transfer and fomite transfer (e.g. footwear and equipment).
- The PEQ facility must be constructed so that it can be cleaned and disinfectant applied effectively and must be maintained in good order.
- The PEQ facility must have facilities for fish examination and parasite treatment.

### Operation

Requirements that apply to the operation of the PEQ facility include:

- 1) All PEQ operations and procedures must be detailed in Standard Operating Procedures (SOPs), consistent with a risk-based approach and approved by the CA of the exporting country.
- 2) PEQ must be under the supervision of the certifying official.
- 3) All equipment used in feeding, handling and treating sturgeon in PEQ must be new or cleaned and disinfected before entry and must be used only in the facility during PEQ.
- 4) During PEQ, the sturgeon of the export consignment must not come into contact with fish of a lesser health status.
- 5) All visits, health problems, tests, test results, treatments, and reasons for removal from the PEQ facility of any sturgeon must be recorded.
- 6) A detailed health record must be kept for each consignment of sturgeon and be available to the certifying official and to the department on request.
- 7) Sturgeon that leave the facility during the PEQ period for any reason cannot re-join the consignment during PEQ.

### Pre-export quarantine period

- 1) The sturgeon must be held in PEQ for at least 30 days and isolated from all other fish not eligible for export to Australia.
- 2) The PEQ period commences from the time the last sturgeon in the export consignment has entered the PEQ facility and the sturgeon have been inspected by the certifying official or a person authorised by the certifying official.

- 3) During the PEQ period, the sturgeon must be observed for signs of infectious disease, parasites or mortalities.
- 4) During the PEQ period, any mortalities must have a full necropsy with results recorded and available to the department.
- 5) During the PEQ period, the sturgeon must undergo parasite treatment(s) within 7 days prior to export if being treated.

### 20.2.3 Certification

The certifying official must certify:

- 1) All sturgeon in the consignment have been resident in an aquaculture premises that has a documented health monitoring program and under the supervision of the CA since birth or for at least 6 months prior to export.
- 2) There has been no significant disease occurrences or parasite infestation in the fish held in the aquaculture premises during the 6 months prior to export.
- 3) The sturgeon have been sourced from premises that only culture sturgeon species.
- 4) During pre-export quarantine:
  - a) The sturgeon were held PEQ for at least 30 days and isolated from all other fish not eligible for export to Australia.
  - b) The sturgeon were inspected by the certifying official within 7 days prior to export and showed no clinical signs of infectious disease or parasites.
- 5) All these biosecurity measures apply:
  - a) typical *Aeromonas salmonicida* freedom
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of typical *Aeromonas salmonicida* (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent).
  - b) *Argulus alosae*, *Argulus coregoni*, *Argulus flavescens*, *Argulus foliaceus*, *Argulus stizostethii*
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of *Argulus alosae*, *Argulus coregoni*, *Argulus flavescens*, *Argulus foliaceus*, *Argulus stizostethii* (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent) **OR**
    - ii) The sturgeon have been treated with an effective parasiticide (e.g. trichlorfon, formaldehyde, sodium chloride) while in PEQ and within 7 days prior to export to Australia to eliminate infestation with *Argulus alosae*, *Argulus coregoni*, *Argulus flavescens*, *Argulus foliaceus*, *Argulus stizostethii* (the active ingredients and concentration must be recorded on the health certificate).
  - c) Cyprinid herpesvirus 3 (CyHV-3) freedom
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of CyHV-3 (freedom would need to be to a standard consistent with that recommended by WOA or equivalent).
  - d) *Ergasilus sieboldi*

- i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of *Ergasilus sieboldi* (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent) **OR**
    - ii) The sturgeon have been treated with an effective parasiticide (e.g. trichlorfon, formaldehyde, sodium chloride) while in PEQ and within 7 days prior to export to Australia to eliminate infestation with *Ergasilus sieboldi* (the active ingredients and concentration must be recorded on the health certificate).
  - e) Frog virus 3 (FV3) freedom
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of FV3 (freedom would need to be to a standard consistent with that recommended by WOA or equivalent).
  - f) Infectious haematopoietic necrosis virus (IHNV) freedom
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of IHNV (freedom would need to be to a standard consistent with that recommended by WOA or equivalent).
  - g) *Polypodium hydriforme* freedom
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of *Polypodium hydriforme* (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent).
  - h) Spring viraemia of carp virus (SVCV) freedom
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of SVCV (freedom would need to be to a standard consistent with that recommended by WOA or equivalent).
  - i) Viral haemorrhagic septicaemia virus (VHSV) freedom
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of VHSV (freedom would need to be to a standard consistent with that recommended by WOA or equivalent).
  - j) *Yersinia ruckeri* (Hagerman strain) freedom
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of *Yersinia ruckeri* (Hagerman strain) (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent).
- 6) All these biosecurity measures may apply (alternative option to post-arrival batch testing):
- a) Acipenserid herpesvirus 1 (AciHV1) and Acipenserid herpesvirus 2 (AciHV2)
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of AciHV1 and AciHV2 (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent).
  - b) Sturgeon nucleocytoplasmic large DNA viruses (sNCLDV)
    - i) The sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of sNCLDV (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent).

- 7) The sturgeon were sealed in its box or carton with tamper-evident seals before leaving the PEQ facility for the port of export and the seal number is recorded on the health certificate.

#### **20.2.4 Transport**

- 1) All sturgeon in the consignment must be packaged in accordance with International Air Transport Association (IATA) Live Animal Regulations.
- 2) All sturgeon in the consignment must be packaged in leak-proof bags with each bag containing only one species of sturgeon. The bag must be colourless and sufficiently transparent to enable proper inspection and identification of the sturgeon and must not contain any extraneous matter, plant material or pests. The bag must be able to withstand the weight of the sturgeon and water and to ensure their safety during transportation. 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.
- 3) 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.
- 4) The consignment must be accompanied by health certification and documents that include the identification number of each box or carton, and the scientific name and number of the contained sturgeon.
- 5) Each bag must be stocked at a density that will allow inspection and must not be overcrowded. When packed for export, sturgeon must be placed in clean water. The use of a pH indicator in the water is permissible, provided it does not interfere with inspection.
- 6) Sturgeon must remain isolated from all animals except those that meet all the conditions described in these biosecurity measures, during transport from the PEQ facility until arrival in Australia.
- 7) Containers used for the transport of sturgeon shall be thoroughly cleansed and disinfected before use.
- 8) Detailed travel plans for transporting the sturgeon to Australia, including contingency plans, must be in place prior to export.
- 9) Boxes or cartons in transit in which there are sturgeon must not be opened unless the Aquatic Animal Health Service of the transit country consider it necessary. If this is the case:
  - a) boxes and cartons shall be subject to precautions to prevent contamination and substitution
  - b) boxes and cartons must only be opened by a certifying official
  - c) boxes and cartons must be resealed with official tamper-evident seals
  - d) it must be documented who opened the box and carton and when and where that occurred and the record sent on with the consignment.
- 10) After the sturgeon arrive at an Australian airport, they must be moved directly to a location specified by the department for inspection (either a regional office, cargo terminal operator or Approved Arrangement (AA)), depending on operational feasibility.

### 20.2.5 On-arrival inspection

The sturgeon will be inspected on arrival at a location specified by the department to ensure that:

- they are healthy
- the health certification meets the import permit conditions
- they are an approved species
- they do not contain non-permitted material or material of biosecurity concern.

Sturgeon not meeting these criteria and non-permitted material will be exported or destroyed at the importer's expense.

Sturgeon meeting these criteria will then be moved directly to the nominated Approved Arrangement (AA) site to undergo post-arrival quarantine (PAQ).

Some mortalities may be present in an otherwise healthy consignment due to the stress of transport. In this case, the sturgeon can still be moved to the AA site where the dead fish will be collected and stored.

Any significant event occurring during transport of the sturgeon to the AA site (e.g. accidents, loss of fish and loss of water) must be reported to the department within 2 hours of discovery of the event.

### 20.2.6 Post-arrival quarantine requirements

#### Location

The AA site must be located within a secure part of an aquaculture facility approved under relevant Australian state or territory legislation to hold sturgeon.

#### Facilities

The facility must meet the department requirements of a new class AA site that is being developed specifically for the import of live sturgeon for aquaculture.

#### Operation

- 1) The AA site must be registered and approved by the department before the required import permit can be assessed.
- 2) All PAQ operations and procedures must follow those outlined, for the yet to be determined AA class, but will include:
  - a) Cleaning and disinfection must occur prior to the arrival of the sturgeon consignment.
  - b) The PAQ period will commence from the time of entry of the sturgeon into the AA site.
  - c) All biosecurity risk material from the consignment (e.g. transport water, bags, boxes and waste material) must be disposed of as biosecurity waste.
  - d) If there is >5% mortality during PAQ, the department must be notified within 24 hours and the sturgeon must undergo investigation to determine the cause of death.
  - e) The department must be notified within 24 hours of any disease incident and its outcome.
  - f) Sturgeon subject to biosecurity control must not leave the AA site during PAQ without written permission from the department.

### Post-arrival quarantine period

During the PAQ period:

- 1) A minimum PAQ period of 30 days applies. The imported sturgeon must remain in the AA site until an age when the species can be visually confirmed. Any variation from the PAQ requirements must be specifically authorised by the department.
- 2) The imported sturgeon must be stocked at high densities (e.g. >13 g fish/L of water) to create the necessary stressor to induce disease in subclinically infected fish (Drennan et al. 2005).
- 3) Any dead sturgeon on arrival or during the PAQ period must be collected and stored in a freezer.
- 4) All imported sturgeon must be observed and inspected daily for clinical signs of significant infectious disease, parasites and mortalities.
- 5) In the event of any imported sturgeon showing clinical signs of an infectious disease or parasite or producing a positive result to any tests indicating the presence of an infectious disease agent, the department may require any or all of the sturgeon in the AA site to remain under biosecurity control. The department will determine the required course of action which may include extending the PAQ period, testing and/or treatment or destruction at the importer's expense.
- 6) At least 10 days after commencement of the PAQ period, the imported sturgeon must be sampled by staff at the AA site, under supervision by the department, and the samples sent to the Australian Centre of Disease Preparedness (ACDP) for batch testing and be found negative for:
  - a) AciHV1 and AciHV2 (required if sturgeon were not certified as free of AciHV1 and AciHV2)
  - b) typical *Aeromonas salmonicida*
  - c) CyHV-3
  - d) FV3
  - e) IHNV
  - f) SVCV
  - g) sNCLDV (required if sturgeon were not certified as free of sNCLDV)
  - h) VHSV
  - i) *Yersinia ruckeri* (Hagerman strain).

(A batch is an epidemiological unit, which means a group of fish that share approximately the same risk of exposure to a disease agent with a defined location. This may be because they share a common aquatic environment (e.g. fish in a pond, caged fish in a lake), or because management practices make it likely that a disease agent in one group of animals would quickly spread to other animals (e.g. all the ponds on a farm, all the ponds in a village system)) (WOAH 2022a).

- 7) All imported sturgeon must be treated with an effective parasiticide (e.g. trichlorfon, formaldehyde, sodium chloride) to eliminate infestation by *Argulus alosae*, *Argulus coregoni*, *Argulus flavescens*, *Argulus foliaceus*, *Argulus stizostethii* and *Ergasilus sieboldi* using a dose and duration to be determined by the department.

- 8) Dead sturgeon during the PAQ period must be sampled by staff at the AA site, under supervision by the department, and the samples sent to ACDP for necroscopy and batch testing for the hazards listed in step 6.
- 9) At the end of the PAQ period, the sturgeon must meet all import conditions before being released from biosecurity control including being free from clinical signs of disease and parasites.
- 10) The department will issue a biosecurity direction to the AA site advising that the sturgeon can be released from biosecurity control.
- 11) The sturgeon must enter a secure recirculating aquaculture system approved by the appropriate state or territory governments as per the import requirements under the *Environment Protection and Biodiversity Conservation Act 1999*.

## **20.3 Proposed biosecurity measures for the importation of sturgeon reproductive material for aquaculture**

### **20.3.1 Documentation**

Importers must obtain a permit from the department to import sturgeon reproductive material into Australia for aquaculture purposes before the reproductive material is imported.

Importers will also require a CITES permit and will need to submit an application through the [Department of Climate Change, Energy, the Environment and Water](#).

Each consignment of sturgeon reproductive material must travel with an original international health certificate that the department has agreed to, conforms to Chapter 5.11. of the WOH Code and is signed by the certifying official of the country of export.

A certifying official means a person authorised by the CA of the country of export to sign health certificates for aquatic animals in accordance with the Certification Procedures of Chapter 5.2 of the WOH Code.

The health certificate must:

- be written in English and a language understood by the certifying official of the country of export
- meet the requirements of [section 20.3.2 Certification](#) and state that all the biosecurity measure requirements have been met
- provide the date(s) of the collection period(s) from each donor sturgeon, the number of eggs/fertilised eggs/milt straws in the consignment for each donor and the means to verify the identification of the reproductive material with the identification details of the donor
- include the name and address of the aquaculture facility of origin
- include the name and address of the exporter and importer and identify the import permit against which it was issued.

The certifying official must:



- provide a health certificate that is specific to the consignment of reproductive material that it covers
- sign, date and stamp (with the stamp of the CA) each page of the health certificate and all attached documents that form part of the extended health certification
- sign and stamp any manual deletions to the health certificate
- record their name, signature and official contact details on the health certificate.

### 20.3.2 Certification

The certifying official must certify:

- 1) All donor sturgeon must be resident in an aquaculture premises that has a documented health monitoring program and under the supervision of the CA since birth or for at least 6 months immediately before reproductive material collection.
- 2) There has been no significant disease occurrences or parasite infestation in the sturgeon held in the aquaculture premises during the 6 months prior to reproductive material collection.
- 3) The donor sturgeon have been sourced from premises that only culture sturgeon species.
- 4) All these biosecurity measures apply:
  - a) typical *Aeromonas salmonicida* freedom
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of typical *Aeromonas salmonicida* (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent).
  - b) Cyprinid herpesvirus 3 (CyHV-3) freedom
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of CyHV-3 (freedom would need to be to a standard consistent with that recommended by WOA or equivalent).
  - c) Frog virus 3 (FV3) freedom
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of FV3 (freedom would need to be to a standard consistent with that recommended by WOA or equivalent).
  - d) Infectious haematopoietic necrosis virus (IHNV) freedom
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of IHNV (freedom would need to be to a standard consistent with that recommended by WOA or equivalent).
  - e) *Polypodium hydriforme* freedom
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of *Polypodium hydriforme* (freedom would need to be to a standard consistent with that recommended for WOA listed diseases or equivalent).
  - f) Spring viraemia of carp virus (SVCV) freedom

- i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of SVCV (freedom would need to be to a standard consistent with that recommended by WOAHP or equivalent).
  - g) Viral haemorrhagic septicaemia virus (VHSV) freedom
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of VHSV (freedom would need to be to a standard consistent with that recommended by WOAHP or equivalent).
  - h) *Yersinia ruckeri* (Hagerman strain) freedom
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of *Yersinia ruckeri* (Hagerman strain) (freedom would need to be to a standard consistent with that recommended for WOAHP listed diseases or equivalent).
- 5) All these biosecurity measures may apply (alternative option to post-arrival batch testing):
- a) Acipenserid herpesvirus 1 (AciHV1) and Acipenserid herpesvirus 2 (AciHV2)
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of AciHV1 and AciHV2 (freedom would need to be to a standard consistent with that recommended for WOAHP listed diseases or equivalent).
  - b) Sturgeon nucleocytoplasmic large DNA viruses (SNCLDV)
    - i) The donor sturgeon are sourced from a country, zone or compartment that is recognised by Australia to be free of SNCLDV (freedom would need to be to a standard consistent with that recommended for WOAHP listed diseases or equivalent).
- 6) The donor sturgeon was not under quarantine restriction for the reproductive material collection period or the 90 days immediately prior.
- 7) Donor sturgeon showed no clinical signs of infectious disease on the day(s) of reproductive material collection and for 30 days after.
- 8) The reproductive material was hygienically collected, handled and processed using properly disinfected or sterilised implements.
- 9) Fertilised eggs have undergone a standard disinfection procedure (Bovo et al. 2005b; Drennan et al. 2006; WOAHP 2022a).
- a) For example, 10 minutes or longer in single-use fresh iodophor at a concentration of 100 ppm at a ratio of 1:4 (1 part non-clumped eggs:4 part active iodine) at pH 7.0–7.5 and alkalinity >100mg/L.
- 10) The reproductive material was not removed from containers for further processing unless previously arranged with the department.
- 11) From the collection until export, the reproductive material in this consignment was:
- a) kept in sealed sterile containers (e.g. straws, ampoules or vials) and sufficiently labelled for identification purposes
  - b) stored and transported only with other sturgeon reproductive material collected for export to Australia or of equivalent health status

- c) kept in a secure place within an aquaculture facility under the supervision of the certifying official
  - d) transported in storage or shipping containers that were either new or had been cleaned and disinfected prior to use.
- 12) An official government seal was applied by a certifying official to the storage or shipping container and the number or mark on the seal recorded on the health certificate.

### **20.3.3 Transport**

- 1) The consignment of sturgeon reproductive material must be accompanied by health certification.
- 2) Sturgeon reproductive material must remain isolated from other reproductive material except those that meet all the conditions described in these biosecurity measures, during transport from the aquaculture facility until arrival in Australia.
- 3) Detailed travel plans for transporting the sturgeon reproductive material to Australia, including contingency plans, must be in place prior to export.
- 4) Containers in transit in which there are sturgeon reproductive material must not be opened unless the Aquatic Animal Health Service of the transit country consider it necessary. If this is the case:
  - a) containers shall be subject to precautions to prevent contamination and substitution
  - b) containers must only be opened by a certifying official
  - c) containers must be resealed with official government seals
  - d) it must be documented who opened the container and when and where that occurred and the record sent on with the consignment.
- 5) After the sturgeon reproductive material arrive at an Australian airport, it must be moved directly to a location specified by the department for inspection (either a regional office, cargo terminal operator, or Approved Arrangement (AA)), depending on operational feasibility.

### **20.3.4 On-arrival inspection**

The sturgeon reproductive material will be inspected on arrival at a location specified by the department to ensure that:

- the contained reproductive material matches the health certification
- the health certification meets the import permit conditions.

Sturgeon reproductive material not meeting these criteria and non-permitted material will be exported or destroyed at the importer's expense.

Sturgeon reproductive material meeting these criteria will then be moved directly to the nominated Approved Arrangement (AA) site to undergo post-arrival quarantine (PAQ).

Any significant event occurring during transport of the sturgeon reproductive material to the AA site (e.g. accidents) must be reported to the department within 2 hours of discovery of the event.

### 20.3.5 Post-arrival quarantine requirements

#### Location

The AA site must be located within a secure part of an aquaculture facility approved under relevant Australian state or territory legislation to hold sturgeon.

#### Facilities

The facility must meet the department requirements of a new class AA site that is being developed specifically for live fish for aquaculture.

#### Operation

- 1) The AA site must be registered and approved by the department before the required import permit can be assessed.
- 2) All PAQ operations and procedures must follow those outlined, for the yet to be determined AA class, but will include:
  - a) Cleaning and disinfection must occur prior to the arrival of the sturgeon consignment.
  - b) The PAQ period will commence from the time of entry of the sturgeon reproductive material into the AA site.
  - c) All biosecurity risk material from the consignment (e.g. shipping containers and liquid nitrogen) must be disposed of as biosecurity waste or cleaned and disinfected in accordance with departmental requirements.
  - d) If there is >5% mortality among the sturgeon progeny during PAQ, the department must be notified within 24 hours and the sturgeon progeny must undergo investigation to determine the cause of death.
  - e) The department must be notified within 24 hours of any disease incident and its outcome.
  - f) Sturgeon reproductive material or sturgeon progeny subject to biosecurity control must not leave the AA site during PAQ without written permission from the department.

#### Post-arrival quarantine period

During the PAQ period:

- 1) The sturgeon reproductive material must remain stored in the AA site until used to produce sturgeon progeny.
- 2) Fertilised eggs must undergo a standard disinfection procedure on arrival before placement into incubation devices (Bovo et al. 2005b; Drennan et al. 2006; WOA 2022a).
  - a) For example, 10 minutes or longer in single-use fresh iodophor at a concentration of 100 ppm at a ratio of 1:4 (1 part non-clumped eggs:4 part active iodine) at pH 7.0–7.5 and alkalinity >100mg/L.
- 3) The sturgeon progeny produced from the reproductive material will be grown out until the life stages can be batch tested for hazards and the species can be visually confirmed (e.g. 3 months post-hatch).
- 4) The sturgeon progeny must be stocked at high densities (e.g. >13 g fish/L of water) to create the necessary stressor to induce disease (Drennan et al. 2005).

- 5) The sturgeon progeny must be observed and inspected daily for clinical signs of infectious disease and mortalities.
- 6) Any dead sturgeon progeny during the PAQ period must be collected and stored in a freezer.
- 7) In the event of any sturgeon progeny showing clinical signs of an infectious disease or producing a positive result to any tests indicating the presence of an infectious disease agent, the department may require any or all the sturgeon progeny in the AA site to remain under biosecurity control. The department will determine the required course of action which may include extending the PAQ period, testing and/or treatment or destruction at the importer's expense.
- 8) The sturgeon progeny must be sampled by staff at the AA site, under supervision by the department, and the samples sent to the Australian Centre of Disease Preparedness (ACDP) for batch testing and be found negative for:
  - a) AciHV1 and AciHV2 (required if donor sturgeon were not certified as free of AciHV1 and AciHV2)
  - b) typical *Aeromonas salmonicida*
  - c) CyHV-3
  - d) FV3
  - e) IHNV
  - f) SVCV
  - g) sNCLDV (required if donor sturgeon were not certified as free of sNCLDV)
  - h) VHSV
  - i) *Yersinia ruckeri* (Hagerman strain).

(A batch is an epidemiological unit, which means a group of fish that share approximately the same risk of exposure to a disease agent with a defined location. This may be because they share a common aquatic environment (e.g. fish in a pond, caged fish in a lake), or because management practices make it likely that a disease agent in one group of animals would quickly spread to other animals (e.g. all the ponds on a farm, all the ponds in a village system)) (WOAH 2022a).

- 9) Dead sturgeon progeny must also be sampled by staff at the AA site, under supervision by the department, and the samples sent to ACDP for necroscopy and batch testing for the hazards listed in step 8.
- 10) At the end of the PAQ period, the sturgeon progeny must meet all import conditions before being released from biosecurity control including being free from clinical signs of disease.
- 11) The department will issue a biosecurity direction to the AA site advising that the sturgeon progeny can be released from biosecurity control.
- 12) The sturgeon progeny must enter a secure recirculating aquaculture system approved by the appropriate state or territory governments as per the import requirements under the *Environment Protection and Biodiversity Conservation Act 1999*.

## **20.4 Verification and compliance activities**

Australia can implement verification activities at the border to provide additional assurances that the certified conditions associated with imports are valid and meet biosecurity requirements. The department also undertakes targeted interventions and time-limited verification activities as deemed necessary. Non-routine interventions are a matter for the department's compliance and enforcement area and will consider information about importer behaviours and other intelligence.

## **20.5 Review of processes**

### **20.5.1 Audit of protocol**

Before the commencement of trade, the department may visit areas in countries that produce live sturgeon for export to Australia. They will evaluate the CA's ability to regulate sturgeon production systems, including systems of health surveillance for Australia's diseases of concern.

### **20.5.2 Review of policy**

The department can review the import policy at any time.

# Appendix A: Australia's regulatory control system for finfish health in Australia

## Australia's finfish disease reporting system

Australia's passive surveillance system underpins early disease detection mechanisms and is used to meet international reporting requirements and provides information to demonstrate Australia's freedom from specific aquatic animal diseases. The system is supported by resources to improve recognition of significant diseases, legal requirements to report notifiable diseases, requirements for aquatic animal producers to report unusual mortality events and a national system to collate information on the occurrence of diseases listed on [Australia's national list of reportable diseases of aquatic animals](#).

Australia has both international and domestic reporting obligations in the event of an aquatic animal disease outbreak – reporting to worldwide, regional, national and state organisations. Australia has reporting requirements to the World Organisation of Animal Health (WOAH) and Network of Aquaculture Centres in the Asia-Pacific (NACA). Australian states or territories also have domestic reporting requirements for notifiable diseases within that jurisdiction.

Each state or territory has their own list of prescribed aquatic animal diseases but must include all aquatic diseases reportable on the national list and their own requirements for reporting and include penalties for failure to report.

## Fish disease surveillance and monitoring in Victoria

Victorian production from wild fisheries (including fish caught in Commonwealth-controlled offshore waters) and aquaculture was valued at just above \$96 million during 2020–21 (Tuynman & Dylewski 2022).

Main fisheries species caught include sardines and abalone.

The main freshwater aquaculture enterprise involves farming of Atlantic salmon, rainbow trout, and barramundi. The main marine aquaculture enterprises involve the farming of abalone and blue mussels.

### Legislation

Legislation governing the fisheries and aquaculture industry in Victoria can be found [here](#).

### Notifiable diseases

Victoria's list of notifiable aquatic diseases can be found [here](#).

### Disease zoning

There have been no finfish disease zones established in Victoria.

## **Fish disease diagnostic services**

Information regarding Victoria's fish disease, and veterinary, diagnostic services can be found [here](#).

## **Fish disease surveillance and monitoring**

### **Fish kill investigations**

Victoria conducts fish kill investigations. Further information can be found [here](#).

## **Domestic movement controls for finfish**

Information on Victoria's translocation control for finfish can be found [here](#).

## **Significant finfish disease agents detected in Victoria**

Finfish disease agents that have been detected in Victoria include:

- *Aeromonas hydrophila*
- *Aeromonas salmonicida* (atypical strains)
- *Chilodonella cyprinid*
- Cyprinid herpesvirus 2
- Dwarf gourami iridovirus
- Epizootic haematopoietic necrosis
- *Enterococcus seriolicida*
- *Flavobacterium columnare*
- *Goussia* species
- *Kudoa thyrsites*
- *Ichthyophthirius multifiliis*
- *Mycobacteria* species
- *Myxobolus gadopsi*
- *Nocardia* species
- Pilchard herpes virus
- Reovirus
- *Saprolegnia* species
- *Triangula percae*
- *Trichodina* species
- *Yersinia ruckeri* (non-Hagerman strains).



## Fish disease surveillance and monitoring in New South Wales

New South Wales (NSW) production from wild fisheries (including fish caught in Commonwealth-controlled offshore waters) and aquaculture was valued at just above \$189 million during 2020–21 (Tuynman & Dylewski 2022).

Main fisheries species caught include sea mullet, rock lobster and king prawns.

The main freshwater aquaculture enterprise involves farming of rainbow trout, barramundi, golden perch, Murray cod, silver perch and yabbies. The main marine aquaculture enterprises involve the farming of oysters and black tiger prawns.

### Legislation

Legislation governing the fisheries and aquaculture industry in NSW can be found [here](#).

### Notifiable diseases

Notifiable aquatic diseases in NSW can be found [here](#).

### Disease zoning

There have been no finfish disease zones established in NSW.

### Fish disease diagnostic services

Information regarding fish disease diagnostic services in NSW can be found [here](#).

### Fish disease surveillance and monitoring

Information regarding NSW's surveillance of disease agents can be found [here](#).

### Fish kills investigations

NSW conducts fish kill investigations. Further information can be found [here](#).

### Domestic movement controls for finfish

Information on the movement of barramundi into NSW can be found [here](#).

### Significant finfish disease agents detected in New South Wales

Finfish disease agents include:

- *Aeromonas hydrophila*
- *Aeromonas salmonicida* (atypical strains)
- *Anasakis* species
- *Aphanomyces invadans*
- *Chilodonella* species
- *Contracaecum* species
- Cyprinid herpesvirus 2
- *Edwardsiella tarda*

- *Eimeria* species
- Epizootic haematopoietic necrosis virus
- Epizootic ulcerative syndrome
- *Flavobacterium columnare*
- *Goussia* species
- *Ichthyobodo necator*
- *Ichthyophthirius multifiliis*
- *Kudoa* species
- *Lactobacillus piscicola*
- Lymphocystis disease
- *Mycobacterium* species
- *Myxobolus* species
- *Nocardia*
- Pilchard herpes virus
- *Saprolegnia* species
- *Streptococcus* species
- *Trichodina* species
- *Vibrio anguillarum*
- *Vibrio cholerae*
- *Vibrio harveyi*
- Viral encephalopathy and retinopathy
- *Yersinia ruckeri* (non–Hagerman strains).

## Fish disease surveillance and monitoring in Tasmania

Tasmanian production from wild fisheries (including fish caught in Commonwealth-controlled offshore waters) and aquaculture was valued at just above \$1.18 billion during 2020–21 (Tuynman & Dylewski 2022).

Main fisheries species caught include abalone and southern rock lobster.

The main freshwater aquaculture enterprise involves farming of salmonids. The main marine aquaculture enterprises involve the farming of salmonids, oysters and abalone.

## Legislation

Legislation governing the fisheries and aquaculture industry in Tasmania can be found [here](#).

## Notifiable diseases

Tasmania's lists of notifiable aquatic diseases can be found [here](#).

## Disease zoning

There have been no finfish disease zones established in Tasmania.

## Fish disease diagnostic services

Information regarding Tasmania's fish disease diagnostic services can be found [here](#).

## Fish disease surveillance and monitoring

### Tasmanian Salmonid Health Surveillance Program (TSHSP)

[TSHSP](#) monitors infectious disease issues for salmonid finfish in Tasmania.

### Investigation of fish kills in Tasmania

Tasmania investigates reported fish kills. Further information can be found [here](#).

### Domestic movement controls for finfish

[Tasmania's Inland Fisheries Service](#) (IFS) oversees the movement of finfish.

### Significant finfish disease agents detected in Tasmania

The significant finfish diseases recorded in Tasmania are:

- *Aeromonas salmonicida* atypical strains (marine aeromonad disease, goldfish ulcer disease)
- Enteric septicaemia of catfish (*Edwardsiella ictaluri*)
- Rickettsia like organism (RLO) of salmonids
- Infection with Pilchard orthomyxo-like virus
- Infection with *Lactococcus garvieae* (Streptococcosis of salmonids)
- Tasmanian aquatic birnavirus.

## Fish disease surveillance and monitoring in Western Australia

Western Australia production from wild fisheries (including fish caught in Commonwealth-controlled offshore waters) and aquaculture was valued at just above \$426 million during 2020–21 (Tuynman & Dylewski 2022).

Main fisheries species caught include western rock lobster, prawns, scallops and snapper.

The main freshwater aquaculture enterprise involves farming of ornamental fish and marron. The main marine aquaculture enterprises involve the farming of pearl oysters and blue mussels.

## Legislation

Legislation governing the fisheries and aquaculture industry in Western Australia can be found [here](#).

## Notifiable diseases

Western Australia's list of notifiable aquatic diseases can be found [here](#).

## Disease zoning

There have been no finfish disease zones established in Western Australia.

## Fish disease diagnostic services

Information regarding Western Australia's fish disease diagnostic services can be found [here](#).

## Fish disease surveillance and monitoring

### Fish kill incident response program

Western Australia responds to reported fish kills. Information can be found [here](#).

## Domestic movement controls for finfish

Western Australia controls the movement of finfish, with some species having specific measures.

Information can be found [here](#).

## Significant finfish disease agents detected in Western Australia

The significant finfish diseases recorded in Western Australia are:

- *Chilodonella hexasticha*
- *Chilodonella cyprini*
- Epitheliocystis
- Epizootic ulcerative syndrome
- *Flavobacterium columnare*
- *Ichthyophthirius multifiliis*
- *Mycobacterium marinum*
- Pilchard herpes virus
- *Trichodina* species
- *Vibrio mimicus*.

## Fish disease surveillance and monitoring in South Australia

South Australian production from wild fisheries (including fish caught in Commonwealth-controlled offshore waters) and aquaculture was valued at just above \$399 million during 2020–21 (Tuynman & Dylewski 2022).

Main fisheries species caught include Australian sardine, King George whiting, mulloway, garfish, yelloweye mullet, southern rock lobster, prawns, and abalone.

The main freshwater aquaculture enterprise involves farming of barramundi, trout, Murray cod marron and yabbies. The main marine aquaculture enterprises involve the farming of wild-caught southern bluefin tuna, yellowtail kingfish, abalone, Pacific oysters and mussels.

## Legislation

The *Livestock Act 1997* includes fish in the definition of livestock; lists notifiable fish diseases; and provides controls over the importation, treatment and movement of farmed fish.

Legislation governing the fisheries and aquaculture industries in South Australia can be found [here](#).

### **Notifiable diseases**

South Australia's notifiable aquatic diseases can be found [here](#).

### **Disease zoning**

There have been no finfish disease zones established in South Australia.

### **Fish disease diagnostic services**

Information regarding South Australia's diagnostic services can be found [here](#).

### **Fish disease surveillance and monitoring**

#### **Mass-die off investigations**

South Australia responds to mass fish die-off events. Information can be found [here](#).

### **Domestic movement controls for finfish**

South Australia regulates the movement of aquatic animals. Information can be found [here](#).

### **Significant finfish disease agents detected in South Australia**

The significant finfish diseases recorded in South Australia are:

- *Aeromonas hydrophila*
- *Acinetobacter haemolyticus*
- amoebae
- *Caligus elongatus*
- *Chilodonella*
- *Enterobacter* species
- Epizootic haematopoietic necrosis
- Epitheliocystis
- *Exophiala salmonis*
- *Flavobacterium* species
- *Ichthyophthirius multifiliis*
- *Klebsiella oxytoca*
- *Kudoa thyrsites*
- Lymphocystis disease
- *Mycobacterium* species
- *Myxobolus* species
- *Photobacterium damsela* subsp. *damsela*
- Pilchard herpes virus

- *Pseudomonas stutzeri*
- *Saprolegnia* species
- *Uronema nigricans*
- *Vibrio vulnificus*
- *Vibrio alginolyticus*
- Viral encephalopathy and retinopathy.

## Fish disease surveillance and monitoring in Queensland

Queensland (QLD) production from wild fisheries (including fish caught in Commonwealth-controlled offshore waters) and aquaculture was valued at just above \$346 million during 2020–21 (Tuyman & Dylewski 2022).

Main fisheries species caught include prawns and coral trout.

The main freshwater aquaculture enterprise involves farming of barramundi, Australian bass, golden perch, jade perch, Murray cod, silver perch, ornamental fish and eels. The main marine aquaculture enterprises involve the farming of barramundi and prawns.

### Legislation

Legislation governing the fisheries and aquaculture industries in QLD can be found [here](#).

### Notifiable diseases

In QLD, all diseases listed in the [Aquatic Animal Diseases Significant to Australia: Identification Field Guide](#) are notifiable.

### Disease zoning

QLD has recognised areas that are free of viral encephalopathy and retinopathy (VER) and the movement of any barramundi into these restricted areas must be certified free of VER before entry.

### Fish disease diagnostic services

QLD's [Biosecurity Sciences Laboratory \(BSL\)](#) conducts aquatic disease diagnostic services.

### Fish disease surveillance and monitoring

#### Fish kill investigation

Significant fish kills in QLD are investigated as pollution incidents. More information can be found [here](#).

#### Targeted surveillance

A 12-month [Surveillance Program for Noxious Fish](#) will commence in August 2023.

### Domestic movement controls for finfish

QLD controls the movement of live aquatic animals within QLD and from interstate. More information can be found [here](#).

### Significant finfish disease agents detected in Queensland

The significant finfish diseases recorded in QLD are:

- *Aeromonas salmonicida* (atypical strains isolated from ulcerated goldfish held at ornamental fish wholesalers)
- Cyprinid herpesvirus 2
- *Chilodonella cyprini*
- *Citrobacter freundii*
- Dwarf gourami iridovirus
- *Edwardsiella ictaluri*
- Enteric septicaemia of catfish
- Epitheliocystis
- Epizootic ulcerative syndrome
- *Exophiala* species
- *Flexibacter marinum*
- *Flavobacterium columnare*
- *Ichthyophthirius multifiliis*
- Lymphocystis disease
- *Mycobacterium* species
- *Streptococcus iniae*
- *Trichodina* species
- *Vibrio harveyi*.

## Fish disease surveillance and monitoring in the Northern Territory

Northern Territory (NT) production from wild fisheries (including fish caught in Commonwealth-controlled offshore waters) and aquaculture was valued just above \$100 million during 2020–21 (Tuynman & Dylewski 2022).

Main fisheries species caught include mud crabs, barramundi and snapper.

The main freshwater aquaculture enterprise involves farming of barramundi. The main marine aquaculture enterprises involve the farming of barramundi and pearl oysters.

### Legislation

Legislation governing the fisheries and aquaculture industries in the NT can be found [here](#).

### Notifiable diseases

In the NT, a notifiable disease means a disease that causes significant mortality or poor health in fish or aquatic life.

## **Disease zoning**

There have been no finfish disease zones established in NT.

## **Fish disease diagnostic services**

[Berrimah Veterinary Laboratory](#) undertakes diagnostic services of aquatic animals for the NT.

## **Fish disease surveillance and monitoring**

### **Fish kill investigations**

NT investigates reports of fish kills. More information can be found [here](#).

## **Domestic movement controls for finfish**

The NT has protocols for the movement of fish for aquaculture. Information can be found [here](#).

## **Significant finfish disease agents detected in the Northern Territory**

The significant finfish diseases recorded in the NT are:

- Epizootic ulcerative syndrome (EUS)
- Lymphocystis disease
- Viral encephalopathy and retinopathy (VER).

## **Fish disease surveillance and monitoring in the Australian Capital Territory**

The Australian Capital Territory (ACT) has no commercial aquaculture or fisheries, but it has a recreational fishery based on introduced species such as trout.

## **Legislation**

Legislation governing fisheries in the ACT can be found [here](#).

## **Notifiable diseases**

The notifiable diseases in the ACT are the same as those listed on [Australia's National list of reportable diseases of aquatic animals](#).

## **Disease zoning**

There are no finfish disease control zones in the ACT.

## **Fish disease diagnostic services**

Because of the small size and population of the ACT, field investigation services are very limited and local veterinary diagnostic facilities do not exist. Consequently, disease problems are referred to the [Elizabeth Macarthur Agricultural Institute](#) in NSW for diagnosis and further investigation.

## **Fish disease surveillance and monitoring**

This is limited to investigation of disease problems as they arise.



## **Australia's national fish diseases laboratory**

The [Australian Centre for Disease Preparedness](#) (ACDP) is Australia's national reference laboratory and is operated by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) based in Geelong, Victoria.

The ACDP Fish Diseases Laboratory (AFDL) provides diagnostic services for exotic and emerging diseases, export certification testing and health surveillance. These are available for finfish, molluscs and crustaceans. The ADFL acts as a national referral laboratory for aquatic animal diseases and is the WOAHP reference laboratory for the epizootic haematopoietic necrosis virus and ranavirus infections.

# Appendix B: Risk assessment values for unrestricted and restricted import of live sturgeon

**Table 33 Risk assessment values for unrestricted risk of import of live sturgeon and with biosecurity measures applied 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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
<i>A. salmonicida</i> (typical strain)	Unrestricted	L	M	L	L	VL	M	L	H	F	C	E	D	C	A	C	H	M	M	VL	M
	Free stock	VL	M	L	VL	VL	M	L	H	F	C	E	D	C	A	C	H	M	L	VL	L
	Free stock and PAQ with testing	N	M	L	N	N	M	L	H	F	C	E	D	C	A	C	H	M	N	N	N
<i>A. alosae</i> , <i>A. coregoni</i> , <i>A. flavescens</i> , <i>A. foliaceus</i> , <i>A. stizostethii</i>	Unrestricted	M	H	M	M	L	H	H	M	E	B	E	D	B	A	C	M	M	M	L	M
	Free stock	VL	H	M	VL	VL	H	H	M	E	B	E	D	B	A	C	M	M	VL	VL	L

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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
	Pre-export parasite treatment	VL	H	M	VL	VL	H	H	M	E	B	E	D	B	A	C	M	M	VL	VL	L
	Free stock/Pre-export parasite treatment and PAQ with parasite treatment	N	H	M	N	N	H	H	M	E	B	E	D	B	A	C	M	M	N	N	N
CyHV-3	Unrestricted	L	L	L	VL	VL	M	M	M	C	E	E	C	D	D	C	M	M	VL	VL	L
	Free stock	VL	L	L	VL	VL	M	M	M	C	E	E	C	D	D	C	M	M	VL	VL	L
	Free stock and PAQ with testing	N	L	L	N	N	M	M	M	C	E	E	C	D	D	C	M	M	N	N	N
<i>E. sieboldi</i>	Unrestricted	M	H	M	M	L	H	H	M	E	B	E	D	A	A	C	M	M	M	L	M
	Free stock	VL	H	M	VL	VL	H	H	M	E	B	E	D	A	A	C	M	M	VL	VL	L
	Pre-export parasite treatment	VL	H	M	VL	VL	H	H	M	E	B	E	D	A	A	C	M	M	VL	VL	L
	Free stock/Pre-export parasite	N	H	M	N	N	H	H	M	E	B	E	D	A	A	C	M	M	N	N	N

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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
	treatment and PAQ with parasite treatment																				
FV3	Unrestricted	M	M	M	L	L	M	H	H	D	F	E	B	B	E	D	H	H	M	M	H
	Free stock	VL	M	M	VL	VL	M	H	H	D	F	E	B	B	E	D	H	H	L	L	M
	Free stock and PAQ with testing	N	M	M	N	N	M	H	H	D	F	E	B	B	E	D	H	H	N	N	N
IHN	Unrestricted	L	M	L	L	VL	M	L	H	F	C	E	D	D	A	C	H	M	M	VL	M
	Free stock	VL	M	L	VL	VL	M	L	H	F	C	E	D	D	A	C	H	M	L	VL	L
	Free stock and PAQ with testing	N	M	L	N	N	M	L	H	F	C	E	D	D	A	C	H	M	N	N	N
<i>P. hydriforme</i>	Unrestricted	H	M	N	M	N	M	N	L	D	A	D	B	A	A	B	L	N	L	N	L
	Free stock	VL	M	N	VL	N	M	N	L	D	A	D	B	A	A	B	L	N	N	N	N
SVCV	Unrestricted	L	M	L	L	VL	M	H	M	D	E	E	C	D	D	C	M	M	L	VL	L
	Free stock	VL	M	L	VL	VL	M	H	M	D	E	E	C	D	D	C	M	M	VL	VL	L
	Free stock and PAQ with testing	N	M	L	N	N	M	H	M	D	E	E	C	D	D	C	M	M	N	N	N

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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
AciHV1 and AciHV2	Unrestricted	H	H	N	H	N	H	N	L	D	A	D	B	B	A	B	L	N	L	N	L
	Free stock	VL	H	N	VL	N	H	N	L	D	A	D	B	B	A	B	L	N	N	N	N
	PAQ with testing	VL	H	N	VL	N	H	N	L	D	A	D	B	B	A	B	L	N	N	N	N
sNCLDV	Unrestricted	H	H	N	H	N	H	N	L	D	A	D	B	B	A	B	L	N	L	N	L
	Free stock	VL	H	N	VL	N	H	N	L	D	A	D	B	B	A	B	L	N	N	N	N
	PAQ with testing	VL	H	N	VL	N	H	N	L	D	A	D	B	B	A	B	L	N	N	N	N
VHSV	Unrestricted	L	M	L	L	VL	M	M	H	F	D	E	D	D	A	C	H	H	M	L	M
	Free stock	VL	M	L	VL	VL	M	M	H	F	D	E	D	D	A	C	H	H	L	L	M
	Free stock and PAQ with testing	N	M	L	N	N	M	M	H	F	D	E	D	D	A	C	H	H	N	N	N
<i>Y. ruckeri</i> (Hagerman strain)	Unrestricted	M	M	L	L	L	M	M	H	F	C	E	D	C	A	C	H	H	M	M	H
	Free stock	VL	M	L	VL	VL	M	M	H	F	C	E	D	C	A	C	H	H	L	L	M

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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
	Free stock and PAQ with testing	N	M	L	N	N	M	M	H	F	C	E	D	C	A	C	H	H	N	N	N

**Hazards:** *A. salmonicida* (typical strain) = *Aeromonas salmonicida* (typical strain); *A. alosae*, *A. coregoni*, *A. flavescens*, *A. foliaceus*, *A. stizostethii* = *Argulus alosae*, *Argulus coregoni*, *Argulus flavescens*, *Argulus foliaceus*, *Argulus stizostethii*; **CyHV-3** = Cyprinid herpesvirus 3; *E. sieboldi* = *Ergasilus sieboldi*; **FV3** = Frog virus 3; **IHN** = Infectious haematopoietic necrosis; *P. hydriforme* = *Polypodium hydriforme*; **SVCV** = Spring viraemia of carp; **AcHV1 and AcHV2** = Acipenserid Herpesvirus 1 and Acipenserid Herpesvirus 2; **sNCLDV** = Sturgeon nucleocytoplasmic large DNA viruses; **VHSV** = viral haemorrhagic septicaemia virus; *Y. ruckeri* (Hagerman strain) = *Yersinia ruckeri* (Hagerman strain). **Biosecurity measures:** **Unrestricted** = no biosecurity measures applied; **Free stock** = sourcing live sturgeon from disease-free stock; **PAQ with testing** = live sturgeon are held under post-arrival quarantine with batch testing applied. **PEQ parasite treatment** = live sturgeon are held under pre-export quarantine with parasite treatment applied. **PAQ parasite treatment** = live sturgeon are held under post-arrival quarantine with parasite treatment applied. **Risk rating:** **E** Extreme. **H** High. **M** Moderate. **L** Low. **VL** Very low. **EL** Extremely low. **N** Negligible. **NA** Not assessed. **Impact score (A, B, C, D, E, F):** See Figure 5.

# Appendix C: Risk assessment values for unrestricted and restricted import of sturgeon reproductive material

**Table 34** Risk assessment values for unrestricted risk of import of sturgeon reproductive material and with biosecurity measures applied 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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
<i>A. salmonicida</i> (typical strain)	Unrestricted	L	M	L	L	VL	M	L	H	F	C	E	D	C	A	C	H	M	M	VL	M
	Free stock	VL	M	L	VL	VL	M	L	H	F	C	E	D	C	A	C	H	M	L	VL	L
	Free stock and PAQ (with progeny production and testing)	N	M	L	N	N	M	L	H	F	C	E	D	C	A	C	H	M	N	N	N
CyHV-3	Unrestricted	L	L	L	VL	VL	M	M	M	C	E	E	C	D	D	C	M	M	VL	VL	L
	Free stock	VL	L	L	VL	VL	M	M	M	C	E	E	C	D	D	C	M	M	VL	VL	L

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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
	Free stock and PAQ (with progeny production and testing)	N	L	L	N	N	M	M	M	C	E	E	C	D	D	C	M	M	N	N	N
FV3	Unrestricted	M	M	M	L	L	M	H	H	D	F	E	B	B	E	D	H	H	M	M	H
	Free stock	VL	M	M	VL	VL	M	H	H	D	F	E	B	B	E	D	H	H	L	L	M
	Free stock and PAQ (with progeny production and testing)	N	M	M	N	N	M	H	H	D	F	E	B	B	E	D	H	H	N	N	N
IHN	Unrestricted	L	M	L	L	VL	M	L	H	F	C	E	D	D	A	C	H	M	M	VL	M
	Free stock	VL	M	L	VL	VL	M	L	H	F	C	E	D	D	A	C	H	M	L	VL	L
	Free stock and PAQ (with progeny production and testing)	N	M	L	N	N	M	L	H	F	C	E	D	D	A	C	H	M	N	N	N



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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
<i>P. hydriforme</i>	Unrestricted	H	M	N	M	N	M	N	L	D	A	D	B	A	A	B	L	N	L	N	L
	Free stock	VL	M	N	VL	N	M	N	L	D	A	D	B	A	A	B	L	N	N	N	N
SVCV	Unrestricted	L	M	L	L	VL	M	H	M	D	E	E	C	D	D	C	M	M	L	VL	L
	Free stock	VL	M	L	VL	VL	M	H	M	D	E	E	C	D	D	C	M	M	VL	VL	L
	Free stock and PAQ (with progeny production and testing)	N	M	L	N	N	M	H	M	D	E	E	C	D	D	C	M	M	N	N	N
AciHV1 and AciHV2	Unrestricted	H	H	N	H	N	H	N	L	D	A	D	B	B	A	B	L	N	L	N	L
	Free stock	VL	H	N	VL	N	H	N	L	D	A	D	B	B	A	B	L	N	N	N	N
	PAQ (with progeny production and testing)	VL	H	N	VL	N	H	N	L	D	A	D	B	B	A	B	L	N	N	N	N
sNCLDV	Unrestricted	H	H	N	H	N	H	N	L	D	A	D	B	B	A	B	L	N	L	N	L
	Free stock	VL	H	N	VL	N	H	N	L	D	A	D	B	B	A	B	L	N	N	N	N

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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species	All species
	PAQ (with progeny production and testing)	VL	H	N	VL	N	H	N	L	D	A	D	B	B	A	B	L	N	N	N	N
VHSV	Unrestricted	L	M	L	L	VL	M	M	H	F	D	E	D	D	A	C	H	H	M	L	M
	Free stock	VL	M	L	VL	VL	M	M	H	F	D	E	D	D	A	C	H	H	L	L	M
	Free stock and PAQ (with progeny production and testing)	N	M	L	N	N	M	M	H	F	D	E	D	D	A	C	H	H	N	N	N
<i>Y. ruckeri</i> (Hagerman strain)	Unrestricted	M	M	L	L	L	M	M	H	F	C	E	D	C	A	C	H	H	M	M	H
	Free stock	VL	M	L	VL	VL	M	M	H	F	C	E	D	C	A	C	H	H	L	L	M
	Free stock and PAQ (with progeny)	N	M	L	N	N	M	M	H	F	C	E	D	C	A	C	H	H	N	N	N

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
			Farmed species	Wild species	Farmed species	Wild species	Farmed species	Wild species	Total	Direct – animal health	Direct – environment	Indirect – control costs	Indirect – domestic trade	Indirect – international trade	Indirect – environment	Indirect -social	Farmed species	Wild species	Farmed species	Wild species
	production and testing)																			

**Hazards:** *A. salmonicida* (typical strain) = *Aeromonas salmonicida* (typical strain); **CyHV-3** = Cyprinid herpesvirus 3; **FV3** = Frog Virus 3; **IHN** = Infectious haematopoietic necrosis; *P. hydriforme* = *Polypodium hydriforme*; **SVCV** = Spring viraemia of carp virus; **AcHV1 and AcHV2** = Acipenserid Herpesvirus 1 and Acipenserid Herpesvirus 2; **sNCLDV** = Sturgeon nucleocytoplasmic large DNA viruses; **VHSV** = viral haemorrhagic septicaemia virus; *Y. ruckeri* (Hagerman strain) = *Yersinia ruckeri* (Hagerman strain). **Biosecurity measures:** **Unrestricted** = no biosecurity measures applied; **Free stock** = sourcing sturgeon reproductive material from disease-free stock; **PAQ (with progeny production and testing)** = on arrival, sturgeon reproductive material is held under post-arrival quarantine for progeny production and resulting larvae/juveniles are batch tested. **Risk rating:** **E** Extreme. **H** High. **M** Moderate. **L** Low. **VL** Very low. **EL** Extremely low. **N** Negligible. **NA** Not assessed. **Impact score (A, B, C, D, E, F):** See Figure 5.

# Appendix D: Biosecurity measures to manage the risk of hazards in other imports to Australia

**Table 35 Biosecurity measures to manage the risk of hazards in other imports to Australia**

Hazard	Biosecurity measures		
	Ornamental fish for display purposes	Non-viable salmonids, and non-salmonid marine finfish for human consumption	Bony fish and cephalopods for aquaculture, bait or pet food use
Typical <i>Aeromonas salmonicida</i>	<p><i>Carassius auratus</i> (goldfish) imported to Australia for display purposes must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>originate from a country, zone or export premises (the population) determined to be free from <i>Aeromonas salmonicida</i> based on: <ul style="list-style-type: none"> <li>the absence of clinical, laboratory or epidemiological evidence of these agents in the source fish population in the previous 2 years, and</li> <li>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</li> </ul> </li> <li>have been inspected within 7 days prior to export and show no clinical signs of infectious disease</li> <li>have been in approved export premises for 14 days prior to export</li> <li>have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing) or koi carp</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p> <ul style="list-style-type: none"> <li>be inspected on arrival in Australia and moved directly to an approved arrangement site and remain for a minimum of 21 days.</li> </ul>	<p>Non-viable salmonids, and non-salmonid marine finfish imported to Australia for human consumption must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>be derived from a population for which there is a documented system of health surveillance and monitoring administered by a competent authority</li> <li>not be derived from a population slaughtered as an official disease control measure</li> <li>not be juvenile salmonids or sexually mature fish (spawners)</li> <li>have had the head and gills removed and internal and external surfaces thoroughly washed to remove any extraneous material</li> <li>be inspected and graded under the supervision of a competent authority</li> <li>be free from visible lesions associated with infectious disease and fit for human consumption</li> <li>be processed in a premises approved by and under the control of a competent authority</li> <li>be exported to Australia accompanied by official certification confirming that the exported fish meet Australia's import conditions in full.</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p> <ul style="list-style-type: none"> <li>Only premises approved by the department will be permitted to commercially process imported salmonids in Australia</li> </ul>	Not applicable

Hazard	Biosecurity measures		
	Ornamental fish for display purposes	Non-viable salmonids, and non-salmonid marine finfish for human consumption	Bony fish and cephalopods for aquaculture, bait or pet food use
		<ul style="list-style-type: none"> <li>Only consumer-ready product will be released from biosecurity control.</li> </ul>	
<i>Argulus coregoni</i> <i>Argulus foliaceus</i>	<p><i>Carassius auratus</i> (goldfish) imported to Australia for display purposes must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>have been inspected within 7 days prior to export and show no clinical signs of infectious disease or pests</li> <li>have been in approved export premises for 14 days prior to export</li> <li>have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing) or koi carp</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p> <ul style="list-style-type: none"> <li>be inspected on arrival in Australia and moved directly to an approved arrangement site and remain for a minimum of 7 days</li> <li>be treated with approved chemotherapeutants if parasite presence is confirmed.</li> </ul>	Not applicable	Not applicable
<i>Ergasilus sieboldi</i>	<p><i>Carassius auratus</i> (goldfish) imported to Australia for display purposes must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>have been inspected within 7 days prior to export and show no clinical signs of infectious disease or pests</li> <li>have been in approved export premises for 14 days prior to export</li> <li>have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing) or koi carp</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p>	Not applicable	Not applicable

Hazard	Biosecurity measures		
	Ornamental fish for display purposes	Non-viable salmonids, and non-salmonid marine finfish for human consumption	Bony fish and cephalopods for aquaculture, bait or pet food use
	<ul style="list-style-type: none"> <li>be inspected on arrival in Australia and moved directly to an approved arrangement site and remain for a minimum of 7 days</li> <li>be treated with approved chemotherapeutants if parasite presence is confirmed.</li> </ul>		
Megalocytiviruses, another genus in the Iridoviridae family that contains ranaviruses (frog virus 3 the species)	<p>Gouramis, bettas, paradise fish, cichlids, and poeciliids imported to Australia for display purposes must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>have source population freedom               <ul style="list-style-type: none"> <li>the fish originate from a country, zone or export premises determined by the Competent Authority to be free from megalocytiviruses consistent with the procedures described in Additional health certification criteria and procedures for gouramis, bettas, paradise fish, cichlids and poeciliids, exported to Australia <b>or</b></li> </ul> </li> <li>batch test negative               <ul style="list-style-type: none"> <li>the batch of consigned fish have been tested by the Competent Authority and found negative for megalocytiviruses consistent with definitions and testing methodology described in Additional health certification criteria and procedures for gouramis, bettas, paradise fish, cichlids and poeciliids exported to Australia, and</li> </ul> </li> <li>have been inspected within 7 days prior to export and show no clinical signs of infectious disease or pests</li> <li>have been in approved export premises for 14 days prior to export</li> <li>have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing) or koi carp</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p>	Not applicable	Not applicable

Hazard	Biosecurity measures		
	Ornamental fish for display purposes	Non-viable salmonids, and non-salmonid marine finfish for human consumption	Bony fish and cephalopods for aquaculture, bait or pet food use
	<ul style="list-style-type: none"> <li>be inspected on arrival in Australia and moved directly to an approved arrangement site and remain for a minimum of 7 days.</li> </ul>		
Infectious haematopoietic necrosis virus	Not applicable	<p>Non-viable salmonids, and non-salmonid marine finfish imported to Australia for human consumption must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>be derived from a population for which there is a documented system of health surveillance and monitoring administered by a competent authority</li> <li>not be derived from a population slaughtered as an official disease control measure</li> <li>not be juvenile salmonids or sexually mature fish (spawners)</li> <li>have had the head and gills removed and internal and external surfaces thoroughly washed to remove any extraneous material</li> <li>be inspected and graded under the supervision of a competent authority</li> <li>be free from visible lesions associated with infectious disease and fit for human consumption</li> <li>be processed in a premises approved by and under the control of a competent authority</li> <li>be exported to Australia accompanied by official certification confirming that the exported fish meet Australia's import conditions in full.</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p> <ul style="list-style-type: none"> <li>Only premises approved by the department will be permitted to commercially process imported salmonids in Australia</li> <li>Only consumer-ready product will be released from biosecurity control.</li> </ul>	Not applicable
Spring viremia of carp virus (SVCV)	<p><i>Carassius auratus</i> (goldfish) imported to Australia for display purposes must:</p> <p>PRE-EXPORT REQUIREMENTS</p>	Not applicable	Not applicable

Hazard	Biosecurity measures		
	Ornamental fish for display purposes	Non-viable salmonids, and non-salmonid marine finfish for human consumption	Bony fish and cephalopods for aquaculture, bait or pet food use
	<ul style="list-style-type: none"> <li>originate from a country, zone or export premises (the population) determined to be free from SVCV based on               <ul style="list-style-type: none"> <li>the absence of clinical, laboratory or epidemiological evidence of these agents in the source fish population in the previous 2 years, and</li> <li>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</li> </ul> </li> <li>have been inspected within 7 days prior to export and show no clinical signs of infectious disease or pests</li> <li>have been in approved export premises for 14 days prior to export</li> <li>have not been kept in water in common with farmed foodfish (fish farmed for human consumption including recreational fishing) or koi carp</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p> <ul style="list-style-type: none"> <li>be inspected on arrival in Australia and moved directly to an approved arrangement site and remain for a minimum of 21 days.</li> </ul>		
Viral haemorrhagic septicaemia virus	Not applicable	<p>Non-viable salmonids, and non-salmonid marine finfish imported to Australia for human consumption must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>be derived from a population for which there is a documented system of health surveillance and monitoring administered by a competent authority</li> <li>not be derived from a population slaughtered as an official disease control measure</li> <li>not be juvenile salmonids or sexually mature fish (spawners)</li> </ul>	<p>Bony fish imported to Australia for aquaculture and bait use must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>be non-viable</li> <li>be wild caught</li> <li>be processed in premises (including vessels/refrigerated warehouses) approved by and under the control of the competent authority</li> <li>be free from visible lesions associated with infectious disease</li> </ul>



Hazard	Biosecurity measures		
	Ornamental fish for display purposes	Non-viable salmonids, and non-salmonid marine finfish for human consumption	Bony fish and cephalopods for aquaculture, bait or pet food use
		<ul style="list-style-type: none"> <li>have had the head and gills removed and internal and external surfaces thoroughly washed to remove any extraneous material</li> <li>be inspected and graded under the supervision of a competent authority</li> <li>be free from visible lesions associated with infectious disease and fit for human consumption</li> <li>be processed in a premises approved by and under the control of a competent authority</li> <li>be exported to Australia accompanied by official certification confirming that the exported fish meet Australia's import conditions in full.</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p> <ul style="list-style-type: none"> <li>Only premises approved by the department will be permitted to commercially process imported salmonids in Australia</li> <li>Only consumer-ready product will be released from biosecurity control.</li> </ul>	<ul style="list-style-type: none"> <li>be inspected under the supervision of the competent authority and/or systems approved by the competent authority.</li> </ul> <p>POST-ARRIVAL REQUIREMENT</p> <ul style="list-style-type: none"> <li>Frozen blocks can be used at water temperatures equal to or greater than 15°C</li> <li>Bony fish must be thawed prior to use below 15°C</li> <li>Bony fish can be fed out any time of the year above 30°S.</li> </ul>
<i>Yersinia ruckeri</i> (Hagerman strain)	Not applicable	<p>Non-viable salmonids, and non-salmonid marine finfish imported to Australia for human consumption must:</p> <p>PRE-EXPORT REQUIREMENTS</p> <ul style="list-style-type: none"> <li>be derived from a population for which there is a documented system of health surveillance and monitoring administered by a competent authority</li> <li>not be derived from a population slaughtered as an official disease control measure</li> <li>not be juvenile salmonids or sexually mature fish (spawners)</li> <li>have had the head and gills removed and internal and external surfaces thoroughly washed to remove any extraneous material</li> <li>be inspected and graded under the supervision of a competent authority</li> </ul>	Not applicable

Hazard	Biosecurity measures		
	Ornamental fish for display purposes	Non-viable salmonids, and non-salmonid marine finfish for human consumption	Bony fish and cephalopods for aquaculture, bait or pet food use
		<ul style="list-style-type: none"> <li>be free from visible lesions associated with infectious disease and fit for human consumption</li> <li>be processed in a premises approved by and under the control of a competent authority</li> <li>be exported to Australia accompanied by official certification confirming that the exported fish meet Australia's import conditions in full.</li> </ul> <p>POST-ARRIVAL REQUIREMENTS</p> <ul style="list-style-type: none"> <li>Only premises approved by the department will be permitted to commercially process imported salmonids in Australia</li> <li>Only consumer-ready product will be released from biosecurity control.</li> </ul>	

# Glossary

Term	Definition
Appropriate level of protection (ALOP)	The <a href="#">Biosecurity Act 2015</a> 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.
Approved arrangement (AA)	Approved arrangement (AA) is defined in the <i>Biosecurity Act 2015</i> as an arrangement for which an approval is in force under paragraph 406(1)(a) (including a varied arrangement for which an approval is in force under that paragraph as it applies because of subsection 412(3)).
Australian territory	Australian territory as referenced in the <i>Biosecurity Act 2015</i> refers to Australia, Christmas Island and Cocos (Keeling) Islands.
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 import risk analysis (BIRA)	The <i>Biosecurity Act 2015</i> defines a BIRA as an evaluation of the level of biosecurity risk associated with particular goods, or a particular class of goods, that may be imported, or proposed to be imported, into Australian territory, including, if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or the class of goods, to a level that achieves the ALOP for Australia. The risk analysis process is regulated under legislation.
Biosecurity measures	The <i>Biosecurity Act 2015</i> defines biosecurity measures as measures to manage 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 <i>Biosecurity Act 2015</i> 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.
Caviar	Roe from sturgeon species.
Certifying Official	A person authorised by the competent authority to sign health certificates for aquatic animals.
Closed system	A system in which there is good control of both host movement and water flow (e.g. a recirculating aquaculture system).
Compartment	One or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purposes of international trade. Such compartments must be clearly documented by the competent authority.
Competent Authority	The Veterinary Authority or other Governmental 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 Aquatic Code in the whole territory.
Endemic	Belonging to, native to, or prevalent in a particular geography, area or environment.
Epidemiological unit	A group of animals that share approximately the same risk of exposure to a pathogenic agent with a defined location. This may be because they share a common aquatic environment (e.g. fish in a pond, caged fish in a lake) or because management practices make it likely that a pathogenic agent in one group of animals would quickly spread to other animals (e.g. all the ponds on a farm, all the ponds in a village system).
Equivalence	The state wherein the biosecurity measure(s) proposed by the exporting country as an alternative to those of the importing country, achieve(s) the same level of protection as those prescribed by the importing country.

<b>Term</b>	<b>Definition</b>
Exotic	When referring to a disease, is not present in the country of concern, and for which measures are in place to either prevent or detect possible incursion of the disease into the country.
Fomite	An inanimate object or material that, when contaminated with or exposed to infectious agents, can transfer disease to new host (e.g., equipment).
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.
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.
Import permit	Official document authorising importation of a commodity in accordance with specified sanitary import requirements.
Official control program	A program which is approved and managed or supervised by the Veterinary Authority of a Member Country for the purpose of controlling a pathogen or disease by specific measures applied throughout that Member Country, or within a zone or compartment of that Member Country.
Open system	A system in which there is no control of either host movement or water flow (e.g. wild-caught fisheries).
Pathogen	A biological agent that can cause disease to its host.
Polyculture	Where more than one species is grown at the same time and place in the same system
Prevalence	The total number of infected aquatic animals expressed as a percentage of the total number of aquatic animals in a given aquatic animal population at one specific time.
Quarantine	Official confinement of regulated articles for observation and research or for further inspection, testing or treatment.
Reproductive material	Sturgeon milt, unfertilised eggs and fertilised eggs
Restricted risk	Risk estimate with sanitary 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.
Semi-closed system	A system in which there is control of host movement and some control of water flow (e.g. pond culture, race culture).
Semi-open system	A system in which there is control of host movement but no control of water flow (e.g. net-pen culture).
Sensitivity	The proportion of true positive tests given in a diagnostic test, meaning the number of true positive results divided by the number of true positive and false negative results.
Specificity	The probability that absence of infection will be correctly identified by a diagnostic test, meaning the number of true negative results divided by the number of true negative and false positive results.
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures.
Stakeholders	Government agencies, individuals, community or industry groups or organizations, 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 which collects and records data on pathogen occurrence or absence by surveying, monitoring or other procedures.

Term	Definition
Targeted surveillance	Surveillance targeted at a specific disease or infection.
The department	The Australian Government Department of Agriculture, Fisheries and Forestry
Unrestricted risk	Risk estimate without the application of biosecurity measures.
Vector	An organism that does not cause disease itself, but which causes infection by conveying pathogens from one host to another.
Zone	An area in one or more countries containing an aquatic animal population with a specific aquatic animal health status with respect to a disease, in which surveillance and control measures and basic biosecurity conditions are applied. The zone should be defined by the Competent authority.

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