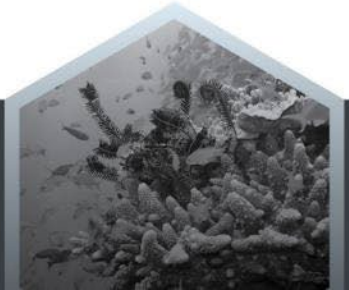
Australian Government

MARINE PESTS

Response manual for invasive marine bivalves

Version 1.0, April 2025

Nationally agreed guidance material endorsed by the Marine Pest Sectoral Committee



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This publication is available at https://www.marinepests.gov.au/what-we-do/emergency/response-manuals

Marine Pest Sectoral Committee Secretariat
Department of Agriculture, Fisheries and Forestry
GPO 858 Canberra ACT 2601
Email mpsc@aff.gov.au
Web www.marinepests.gov.au

Disclaimer

These manuals are part of a series of documents providing detailed information and guidance for emergency response to key marine pest species or groups of marine pest species.

The manuals are made available on the understanding that the Commonwealth of Australia is not thereby engaged in rendering professional advice. The Commonwealth does not warrant the accuracy, currency or completeness of the guidelines, or their relevance for any particular purpose. In particular it should be noted that legislation, regulations and by-laws may vary between different jurisdictions and ports in Australia. Consequently, the guidelines do not purport to state what is necessary or sufficient to comply with laws applying in any place.

Before relying on the manuals in any important matter, users should obtain appropriate professional advice to evaluate their accuracy, currency, completeness, and relevance for their purposes.

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.

Note

Response manuals provide guidance for Australian marine pest biosecurity responses. They provide detailed information and guidance for emergency response to a marine pest incident. The guidance is based on sound analysis and links policy, strategies, implementation, coordination, and emergency management plans.

Preface

The Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) maintains a series of response[1] manuals to ensure national coordination of emergency responses to incursions by exotic pests and diseases or significant range expansions of established pests and endemic diseases. The response manuals for marine pests provide detailed information and guidance for emergency response to key marine pest species or groups of pest species of national significance.

The response manuals are adapted from the Australian emergency plans for terrestrial and aquatic animal diseases—the Australian Veterinary Emergency Plan (AUSVETPLAN) and the Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN). The format and content have been kept as similar as possible to those documents to enable emergency response personnel trained in their use to work efficiently with these manuals in the event of a marine pest emergency.

This manual describes the principles for an emergency response to an incident caused by the suspicion or confirmation of incursion by an invasive marine bivalve.

The National Institute of Water and Atmospheric Research, New Zealand (NIWA), and DAFF, Australia, prepared the first edition of this response manual. It has gone through an extensive process of editing and comment from the Marine Pest Sectoral Committee (MPSC) and relevant experts. The MPSC endorsed this manual on 28 October 2024.

The manual will be reviewed and updated as required to incorporate new information and experience gained with incursion management of these or similar marine pests. Amended versions will be published on the marine pests website.

[1] Note that the term 'emergency response' as used in this document does not refer to a 'biosecurity emergency' as that term is used under the *Biosecurity Act 2015*, nor are any activities described by this document undertaken during an 'emergency response' intended to be an exercise of powers provided by Chapter 8 (Biosecurity Emergencies and Human Biosecurity Emergencies) of that Act.

Recommendations for amendments

To recommend changes or corrections to this document, please forward your suggestions to:

Marine Pest Sectoral Committee Secretariat
Department of Agriculture, Fisheries and Forestry
GPO 858 Canberra City ACT 2601
Email mpsc@aff.gov.au

Proposed changes will be considered by the MPSC before being incorporated into the manual.

Version history

Version	Date	Amendment details
1	March 2025	First publication

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Introduction

Marine pests are non-native marine species introduced to areas outside their native geographic range. They can have negative impacts to Australia's marine environment, social amenity, or marine industries. Preventing new introductions of marine pests is more cost effective than control (Leung et al. 2002). Where introductions occur, both short and long-term impacts and costs can be limited by a rapid and effectively managed response to the incursion (Campbell et al. 2018; Wotton & Hewitt 2004).

Manual purpose

Emergency response operations are most effective if they are based on detailed knowledge of the marine pest's life history, biology and ecology, ability to introduce or carry pathogens, and susceptibility to control measures or eradication. Response actions are most effective when taken immediately (or as soon as possible) after a marine pest incursion is first detected. The purpose of this document is to serve as a reference for managing emergency responses to invasive marine bivalves, and to provide guidance in a response.

Emergency Marine Pest Plan Series

The Marine Pest Response Manuals are a series of guidance documents that provide information on marine pest emergency responses. The response manuals are part of the Emergency Marine Pest Plan (EMPPlan) series, a broader set of guidance documents that inform all aspects of a marine pest response. This manual is one of the response manuals and is intended to be used alongside other documents in the EMPPlan series.

Previously, marine pest response manuals were developed for individual pest species. As part of the EMPPlan series, the manuals have transitioned from focusing on individual pest species to taxonomic groups of invasive marine animals or plants. The taxon-specific manual template consolidates technical and management information on groups of marine pests (e.g. crabs, bivalves, ascidians) into one document to support marine pest response activities.

Species and taxon-specific Marine Pest Response Manuals have been published for several marine pests that the Marine Pest Sectoral Committee (MPSC) has identified as being of national significance:

- Response manual for invasive marine crabs
- Response manual for invasive marine bivalves
- Northern pacific seastar (*Asterias amurensis*)
- Japanese seaweed/Wakame (*Undaria pinnatifida*).

These response manuals offer guidance on the types of information needed to inform the response to a marine pest incursion and appropriate methods of containment, control and/or eradication of marine pest taxa. The manuals may be used when planning for or responding to a suspected or confirmed marine pest incursion.

The Marine pest response manual has also been developed which covers general information on responding to marine pest incursions in the absence of species or taxon-specific manuals.

The Biosecurity Incident Management System (BIMS): Marine pest version provides a uniform approach for managing responses to marine pest biosecurity incidents. It aligns with the response management approach applied to all biosecurity sectors. The manual provides guidance in contemporary practices for the management of marine pest biosecurity incident responses and initial recovery operations in Australia.

The National Introduced Marine Pest Information System (NIMPIS) is the central repository of information on the biology, ecology, and the distribution of over 150 marine pest species either introduced, established, or that pose a risk of future introduction to Australia. NIMPIS is a key source of information on invasive marine bivalves of relevance to Australia. NIMPIS can be used in conjunction with this response manual to provide information on biological and management information relating to invasive marine bivalves in Australia.

Manual format

This response manual describes practical management for an emergency response to an incident caused by the suspicion or confirmation of an incursion by an invasive marine bivalve. The manual is intended to be used in conjunction with appropriate existing Australian Veterinary Emergency Plan (AQUAVETPLAN) manuals, which detail the disposal, destruction, and decontamination for disease control if a disease is introduced with an invasive marine bivalve. The manuals are best used in digital format (e.g. PDF) for ease of navigation between headings, subheadings, and hyperlinks throughout the manual. Users are encouraged to navigate to the relevant sections during an emergency response.

The introduction covers the manual's purpose, format, and scope. It also covers general bivalve biology, including bivalve diseases, and provides a brief overview on the management of invasive marine bivalves.

Outside of the introduction, there are six main sections within this manual and six appendices (Appendices A-F). Appendix A contains taxon-specific information on some invasive marine bivalves to Australia representing infaunal and epifaunal life habits. The species in Appendix A are grouped by family and are listed in alphabetical order for ease of use. This appendix does not cover every invasive marine bivalve to Australia, either established or exotic. The inclusion and exclusion of bivalve taxa covered in this appendix and throughout the manual is explained further under manual scope. Additional taxonomic information can be located on NIMPIS.

The remaining appendices contain information on:

- Policy principles for determining the status of marine pests
- The Australian Government *Biosecurity Act 2015* (hereafter the *Biosecurity Act 2015*)
- Commonwealth, state, and territory legislative powers
- Example methods for detecting invasive marine bivalves using settlement arrays and plankton tows.

Common word definitions are listed in the glossary at the end of this manual.

Manual scope

The scope of this response manual is to provide key information on invasive marine bivalves and how to respond to an incursion of any invasive marine bivalve, including species which may not be listed in this manual. We have identified invasive marine bivalves that are most likely to be considered in an emergency response or ongoing management in Australia and have used these species as examples to demonstrate how to respond to marine bivalves from different functional groups. For example, fouling mussels and oysters on hard substrates (epifaunal), or clams in soft sediment habitats (infaunal). The key bivalve taxa that are used as key examples in this manual include (but are not limited to):

- **Corbulidae:** Asian basket clam (*Potamocorbula amurensis*) and European basket shell (*Varicorbula gibba*)
- **Dreissenidae:** Black-striped false mussel (*Mytilopsis sallei*)
- **Myidae:** Soft-shell clams (*Mya arenaria and Mya japonica*)
- **Mytilidae:** Asian date/bag mussel (*Arcuatula senhousia*), Charru mussel (*Mytella strigata*), New Zealand green-lipped mussel (*Perna canaliculus*), brown mussel (*Perna perna*) and Asian green mussel (*Perna viridis*)
- **Ostreidae:** Suminoe oyster (*Magallana ariakensis*), black scar oyster (*Magallana bilineata*) and Pacific oyster (*Magallana gigas*).

Taxon-specific information on these species is detailed in Appendix A with further technical information available in NIMPIS. These invasive marine bivalves were selected based on their national listing on the Australian Priority Marine Pest List (APMPL) or the Exotic Environmental Pest List (EEPL), being listed on jurisdiction state and territory lists (i.e. noxious species lists, surveillance lists), listed on the CCIMPE trigger list (not publicly available), having an existing National Control Plan, or due to having demonstrated or potential impacts that may affect response strategies and management actions (see Table 1).

Bivalves included in this manual have been identified as having the potential to cause significant negative environmental, economic, social, or cultural impacts should they arrive to Australia. For species which are already established or cultivated, there is national interest to limit their spread and impact within Australia.

This manual does not cover all invasive marine bivalves to Australia, either introduced, established, or exotic, or for those which may have unknown invasion status (e.g. cryptogenic species). NIMPIS is a good resource of information for species not covered in the manual. Other taxa may be identified for inclusion or exclusion and the manual will be updated as required.

We acknowledge some native bivalve species can also exhibit ‘pest’ traits, but these native bivalves are out-of-scope in this manual. In addition, we recognise some invasive marine bivalves may have perceived or actual positive benefits to the surrounding environment (e.g. habitat structure and biodiversity), economy (e.g. cultivation and aquaculture production), or socio-cultural influences

(e.g. recreational harvest), but this manual's purpose is for bivalves which can have a negative impact on the marine environment, industries, or social amenity and elicit an emergency response.

Finally, this manual focuses on invasive marine bivalves. 'Marine bivalves' are defined as species that spend the majority or the entirety of their lifecycle in marine (salinity 33–37 ppt) or brackish (3–35 ppt) waters. These species may have some ability to survive in freshwater (<3 ppt), but brackish/marine waters are required for the species to reproduce and/or for their long-term survival. We recognise that invasive freshwater bivalves exist to which emergency responses have occurred in Australia (i.e. the freshwater gold clam, *Corbicula fluminea*), but freshwater bivalves are out-of-scope in this current manual. Suspected freshwater invasive bivalves should still be reported to the relevant biosecurity agency in the jurisdiction in which they have been found.

Table 1 Invasive marine bivalves included as key examples throughout this manual and their current status in Australia. Taxa are displayed in alphabetical order

Bivalve species and common name	Presence in Australia[e]	Invasion status in Australia	Listed on the APMPL?	Listed on the EEPL?	Other reasons for inclusion	NIMPIS profile link
Arcuatula senhousia (Asian date/bag mussel)	Established (TAS, VIC, WA)	Non-native	No	No	Listed on state/territory noxious species lists; listed on CCIMPE[c] trigger list; has a National Control Plan (NCP)	NIMPIS profile
Magallana ariakensis (Suminoe oyster)	Recorded (QLD) but uncertain	Non-native	No	No	Listed on state/territory noxious species lists; reportable biosecurity matter under QLD Biosecurity Act 2014	n/a
Magallana bilineata (Black scar oyster)	Established (QLD)	Non-native	No	No	Listed on state/territory noxious species lists; reportable biosecurity matter under QLD Biosecurity Act 2014	NIMPIS profile
Magallana gigas (Pacific oyster)	Established, cultivated (NSW, SA, TAS, VIC)	Non-native	No	No	Listed on state/territory noxious species lists	NIMPIS profile
Mya arenaria (Soft-shell clam)	Not recorded	Non-native	No	Yes	Listed on the EEPL[b]; listed on state/territory noxious species lists; listed on CCIMPE[c] trigger list	NIMPIS profile
Mya japonica[d] (Japanese soft-shell clam)	Established (TAS)	Non-native	No	No	Listed on the EEPL[b]; listed on state/territory noxious species lists; listed on CCIMPE[c] trigger list	n/a
Mytella strigata (Charru mussel)	Recorded (NT) but not established	Non-native	Yes	No	Listed on the APMPL[a]; listed on state/territory noxious species lists	NIMPIS profile
Mytilopsis sallei (Black-striped false mussel)	Recorded (NT) but not established	Non-native	Yes	Yes	Listed on the APMPL[a] and EEPL[b]; listed on state/territory noxious species lists; listed on CCIMPE[c] trigger list	NIMPIS profile
Perna canaliculus (New Zealand green-lipped mussel)	Recorded (SA, VIC) but not established	Non-native	Yes	Yes	Listed on the APMPL[a] and EEPL[b]; listed on state/territory noxious species lists	NIMPIS profile
Perna perna (Brown mussel)	Not recorded	Non-native	Yes	Yes	Listed on the APMPL[a] and EEPL[b]; listed on state/territory noxious species lists; listed on CCIMPE[c] trigger list	NIMPIS profile

Perna viridis (Asian green mussel)	Recorded (NT, QLD, WA) but not established	Non-native	Yes	Yes	Listed on the APMPL[a] and EEPL[b]; listed on state/territory noxious species lists; listed on CCIMPE[c] trigger list	NIMPIS profile
Potamocorbula amurensis (Asian basket clam)	Not recorded	Non-native	No	Yes	Listed on the EEPL[b]; listed on state/territory noxious species lists; listed on CCIMPE[c] trigger list	NIMPIS profile
Varicorbula gibba (European basket shell)	Established (TAS, VIC)	Non-native	No	No	Listed on state/territory noxious species lists; listed on CCIMPE[c] trigger list; has a National Control Plan (NCP)	NIMPIS profile

a Australian Priority Marine Pest List (APMPL)

b The National Priority List of Exotic Environmental Pests, Weeds and Diseases (EEPL)

c The Consultative Committee on Introduced Marine Pest Emergencies Trigger List (not publicly available)

d *Mya japonica* was recently confirmed to be separate species to *M. arenaria* and is assessed the same way as *M. arenaria* in this table

e Established = a non-native bivalve which has been introduced to Australia and established a reproductive population; Recorded but not established = a non-native bivalve which has been recorded in Australia, either as a transient detection on a vessel or from a population which died out, but did not establish; Recorded but uncertain = a non-native bivalve which has been recorded in Australia but its status is uncertain; Not recorded = a non-native bivalve which has not been recorded in Australian waters

n/a - Not applicable

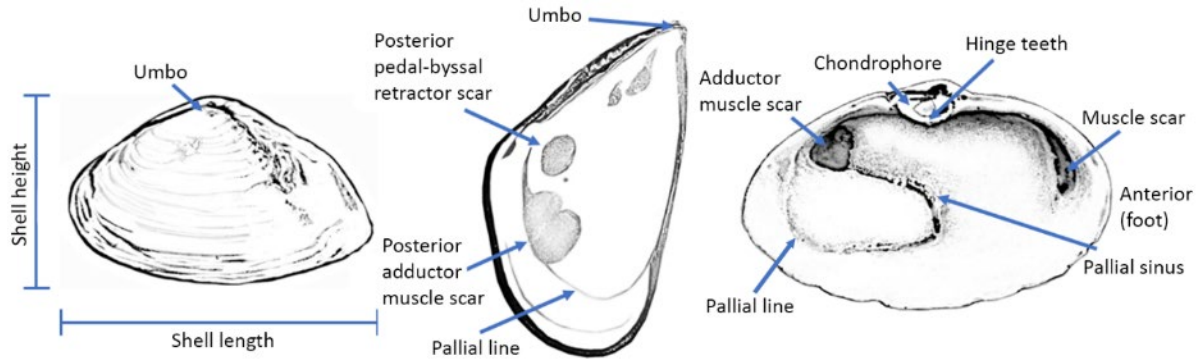
General bivalve biology

Taxonomy

The class Bivalvia belongs to the speciose phylum Mollusca. Bivalves are characterised by a soft body protected by a hard (calcium carbonate) shell divided into two valves and joined by a hinge (Figure 1). The shell is secreted by the mantle which is held closed by adductor muscles with hinge teeth interlocking to prevent the shells from twisting. Bivalve molluscs include common taxa such as clams, oysters, and mussels.

There are more than 100 families and more than 9,000 species of bivalves globally (Huber 2010). They inhabit a broad range of inland waterways, brackish, and marine habitats from the deep sea to high tide level, including rock shores, sandy shores, mudflats, and estuarine areas.

Figure 1 Diagram showing general bivalve shell anatomy



Source: Kimberley Seaward, NIWA

Feeding

Most bivalves are sedentary as adults, but some taxa are motile (e.g. scallops and clams). Most bivalves are suspension feeders, passively feeding by trapping food particles from the surrounding water into their gills. Some other bivalves are deposit feeders (e.g. family Tellinidae) where they feed by ingesting some organic particles within sedimentary deposits. Food consists of microscopic plants, animals, and detritus.

Reproduction, growth, and life cycle

Bivalves can either have separate sexes (male and female gametes) or be hermaphrodites (simultaneously or sequentially possessing both male and female gametes). The cues for changing sex in bivalves are not well understood but they can coincide with environmental conditions, such as food limitation in the case of *Mytella strigata* or age in the case of *Ostrea* spp. *Mytilopsis sallei* can change sex throughout its life so at any time a proportion of the population can be hermaphrodites (Karande & Menon 1975).

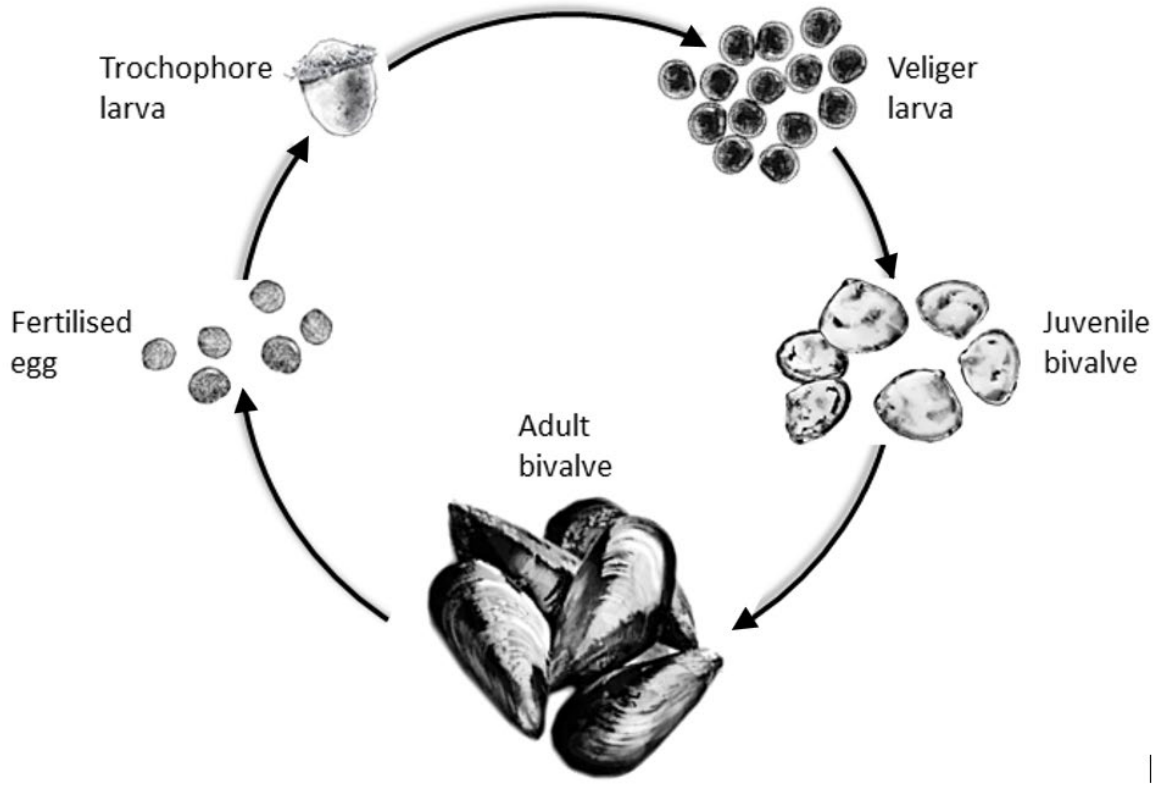
Generally, bivalves are broadcast spawners, releasing sperm and eggs into the water column where external fertilisation takes place. The fertilised egg first develops into trochophore larvae then into veliger larvae. Larvae disperse as plankton, either passively via ocean currents or by active swimming, and can remain in plankton from days to weeks. The veliger larvae settle on the substrata, metamorphose into juveniles, and then grow into adults (Figure 2). Some species, such as flat oysters (genus *Ostrea*) have evolved brooding behaviour, where they draw in water containing

sperm; fertilisation occurs internally and the larvae are retained until they are ready to settle. Non-native *Ostrea* spp. are not regarded as an invasion risk for Australia.

Reproduction is an important factor that influences establishment and spread of an introduced species (Geburzi & McCarthy 2018). Some reproductive traits that may increase invasion success include fecundity, the size at onset of sexual maturity, reproductive/mating strategy, the number, timing, and duration of spawning events, growth rates, and larval duration (Geburzi & McCarthy 2018). Some invasive bivalves are highly fecund, with females capable of releasing tens of thousands of eggs in a single brood. Spawning can occur simultaneously within a population (known as mass spawning) or over a prolonged period with animals releasing sperm and ova continuously for weeks to months. Mass spawning is usually synchronised with changes in the external environment such as water temperature. For example, *Magallana gigas* predominantly spawns when water temperature is over 20°C and rarely when water temperature is under 15°C.

The length of time larvae spend in plankton varies between species and is also influenced by water temperature and feed supply. *Magallana gigas* has a relatively long larval period of around three weeks, *P. amurensis* is around 17 days, whereas *M. sallei* has a comparatively shorter larval phase of around one week. The length of time larvae spend as plankton can impact their ability to spread by some pathways, such as ballast water or ocean currents. For instance, *P. amurensis* is more likely to spread via ballast water over longer distances than *M. sallei* because of its longer larval period.

Figure 2 General lifecycle of marine bivalves



Source: Kimberley Seaward, NIWA

Infaunal bivalves

Bivalves live in a range of marine, brackish, and freshwater environments. As adults they may spend their life buried in marine sediments (infaunal) or on the surface of the seafloor or hard substrata (epifaunal). Most species of marine bivalves are infaunal, living fully or partially buried in, or on the surface of, soft marine substrata such as sand, silt, mud, or gravel. Many infaunal species are parts of intertidal or subtidal communities within estuaries and sheltered bays. There are many infaunal marine bivalves that live in deeper coastal and ocean sediments exceeding 100 m depth or more (e.g. Cuspidariidae, Poromyidae, Bentholyonsiidae, Euciroidae, Lyonsiellidae and Verticordiidae). However, most invasive marine bivalve species tend to be intertidal or shallow-water species (<50 m depth).

Examples of invasive infaunal species include clams, such as *Mya japonica, P. amurensis* and *Varicorbula gibba.* Burying in the sediment is a method that protects the bivalves from predation, desiccation, and unfavourable environments. Infaunal species bury into the sediment using a muscular foot, often positioning themselves vertically so only their siphons are exposed above the sediment, while some families (e.g. family Tellinidae) position themselves obliquely in the sediment. Water sucked into the inhalant siphon carries food and oxygen into the body and waste material is excreted via the exhalent siphon. Some clam species, such as *P. amurensis,* can expose up to half of their body above the sediment surface, whereas some species, like *M. japonica,* can burrow up to 15 cm deep.

Epifaunal bivalves

Epifaunal bivalves are different to infaunal bivalves in that they live on the surface of a substrate, such as hard surfaces, other organisms, or the seafloor. They may be attached or range freely over the surface. Byssus threads are hair- or bristle-like threads which are secreted by the muscular foot of juvenile and adult mussels to attach to surfaces. Examples of invasive epifaunal bivalves which secrete byssus threads include mussels (i.e. *Perna* spp. and *M. strigata*) and false-mussels (e.g. *M. sallei*). *Mytella strigata, Arcuatula senhousia,* and *M. sallei* are epifaunal bivalves which can live both on and within soft sediment.

Oysters are also epifaunal bivalves, but instead of using byssal threads to attach themselves, they cement themselves in place via one of their shell valves. Invasive oyster species include some members of the *Magallana* genus, with some species being widely cultivated (i.e. *M. gigas* and *M. ariakensis*). Bivalves that attach themselves to hard substrata are also referred to as 'fouling' species because they can grow on both natural and manmade substrata and occasionally in high numbers. For example, *M. sallei* can be found in densities exceeding 23,000 per m^2 (Bax et al. 2002). Both native and invasive bivalves can foul structures such as vessel hulls and niche areas, water supply pipes, engine cooling pipes, wharves, pontoons, pylons, buoys, and aquaculture equipment. Invasive marine bivalves can also foul natural structures like rocky shores and reefs and compete with native sessile species for space.

Environmental tolerances and habitat

Bivalves inhabit a broad range of freshwater, brackish, and marine habitats from the deep sea through to the high tide level, including rock shores, sandy shores, mudflats, and estuarine areas. The closed, double shell allows bivalves to withstand emersion, desiccation (air-drying), and unfavourable conditions for periods of time. This can have management implications. For example,

killing invasive bivalves via desiccation can take up to seven days or even longer in some species and the timing of this control method would need consideration.

Knowledge of habitat requirements of an invasive marine bivalve may assist in targeting surveillance within these habitats. For example, clams that are infaunal will usually inhabit soft benthic environments such as mudflats and sandy shores, rather than hard rocky intertidal substrates, whereas mussels and other epifaunal bivalves typically prefer settling on hard structures, such as wharves, pontoons, and vessel hulls.

Bivalves that inhabit intertidal estuarine areas are particularly well adapted to a range of salinity conditions. For example, adult *P. amurensis* can tolerate salinities from 0.1 to 33 ppt, however larval and juvenile stages have a narrower salinity tolerance between 10 and 33 ppt (Nicolini & Penry 2000). Bivalves can also thrive in degraded environments. *Varicorbula gibba* is considered an indicator of environmental degradation caused by pollution, low dissolved oxygen, or increased turbidity because of its ability to survive in such poor conditions. The ability to tolerate low oxygen and low food environments is characteristic of many invasive bivalves. *Mytilopsis sallei* and *P. amurensis* have high tolerances to low oxygen and can live in polluted or eutrophic areas. *Mya arenaria* can survive in an oxygen-free environment for up to 8 days (Cohen 2011) and *M. gigas* can tolerate being out of water for >3 weeks (Atalah et al. 2016). *Perna viridis* is found in hypersaline lagoons (>58 ppt) in Venezuela (Segnini de Bravo et al. 1998). Bivalve larvae are generally more environmentally sensitive than adults but can still have wide environmental tolerances (e.g. *P. amurensis*).

Bivalve diseases

Invasive marine bivalves can introduce pathogens (viruses, bacteria, or parasites) that can cause severe disease, compromising commercial seafood production, impacting natural ecosystems, or potentially effect human health.

Bivalves are susceptible to, or can act as carriers of, a range of molluscan diseases considered significant to Australia. Australia's National List of Reportable Diseases of Aquatic Animals identifies exotic and endemic bivalve diseases that may be spread by both native and invasive bivalves. The Aquatic Animal Diseases Significant to Australia: Identification Field Guide 5th Edition provides further information on bivalve diseases considered significant to Australia. Australia has three high priority diseases that are capable of being introduced or spread with marine bivalves: Pacific oyster mortality syndrome (POMS) caused by ostreid herpesvirus type 1 microvariant (OsHV-1 µvar), bonamiosis caused by haplosporidian parasites (*Bonamia* spp.), and marteiliosis caused by paramyxean parasites (*Marteilia* spp.).

The three high priority bivalve diseases are detailed below; however, it should be noted that not all exotic or endemic bivalve diseases are captured in this section. It is also important to recognise that many native bivalves can also carry or spread disease. Disease needs to be considered following an incursion of an invasive marine bivalve. This includes exotic pathogens that could be introduced with the marine bivalve as well as any decontamination, disposal, or destruction procedures (see Section 6). AQUAVETPLANS and the Aquatic Animal Disease Field Guide contain more detailed information on these diseases and are a primary resource during a marine bivalve pest or disease incursion. It is

recommended that this manual is used in conjunction with available manuals and information on exotic bivalve diseases where possible.

Ostreid herpesvirus-1 microvariant (OsHV-1 µvar)

Infection with ostreid herpesvirus-1 microvariant (OsHV-1 µvar*)* causes POMS and acute mortality in *M. gigas* and *M. angulata*. An Australian AQUAVETPLAN manual exists for OsHV-1 µvar that includes information on the pathobiology, epidemiology, diagnostic methods, and methods to control and eradicate this pathogen in Australia (DAFF 2015).

OsHV-1 µvar has previously been detected in wild *M. gigas* in parts of New South Wales, South Australia, and Tasmania. In 2010, the first POMS outbreak in Australia was detected in the Georges River, New South Wales, with further surveillance detecting it in the Parramatta River, Port Jackson and, in 2013, the Hawkesbury River (Jenkins et al. 2013). In 2011, Tasmania and South Australia were declared free of POMS via surveillance, but the virus was detected in Tasmania in 2016 (de Kantzow et al. 2017) and in South Australia in the Port River in 2018.

In Australia, POMS is primarily a concern for introduced *M. gigas,* which comprise more than 99% of aquaculture production of edible oysters in South Australia and Tasmania. POMS is characterised by a rapid onset of high mortality, up to 100% in infected populations. This can lead to significant production losses for oyster farmers, employment, and business viability. Wild populations of *M. gigas* may act as reservoirs of the disease and present risks to cultivated oysters and farms. It is important to note that this pathogen does not appear to infect native oysters in Australia (e.g. *Saccostrea* or *Ostrea* spp.), or other *Magallana* spp.

Bonamia spp. parasites

Bonamia spp. are haplosporidians, which are parasites of oysters and the causative agent of the disease bonamiasis. *Bonamia* parasites and parasites of the genus *Mikrocytos* are termed microcells because of their small size, typically 1 to 3 µm. An Australian and New Zealand Standard Diagnostic Procedure (ANZSDP) exists for bonamiasis including information on the pathobiology, epidemiology, and diagnostic methods of the parasite in Australia.

Bonamia ostreae is listed as a disease notifiable to the World Organisation of Animal Health (WOAH, founded as OIE) and is also listed on Australia's National List of Reportable Diseases of Aquatic Animals and on the Exotic Environmental Pest List (EEPL). *Bonamia ostreae* is exotic to Australia and has the potential to cause severe disease in susceptible species which include the native Australian flat oyster *Ostrea angasi* (Buss et al. 2020; Engelsma et al. 2014). It has never been recorded in Australia but was recorded in New Zealand in farmed *O. chilensis* for the first time in 2015 (Lane et al. 2016) and is present throughout flat oyster populations of Europe, western and eastern parts of North America, and Morocco (Engelsma et al. 2014). The detection of *M. ariakensis* oysters in Queensland in 2023 raises concerns about the potential introduction of *B. ostreae* to Australia, as microcells are occasionally detected in *M. ariakensis* overseas.

Bonamia exitiosa is related to *B. ostreae* and is primarily a parasite of native flat oysters (*Ostrea* spp.) or rock oysters (*Magallana* spp.) in Australia and can also cause bonamiasis (Buss et al. 2020). Microcells have also been detected in wild *M. gigas. Bonamia exitiosa* is also a WOAH-notifiable disease and is listed on Australia's National List of Reportable Diseases of Aquatic Animals. This parasite has been recorded in flat oysters in Victoria and New South Wales, as well as in a very low

percentage of Sydney rock oysters in New South Wales. It is likely that *Bonamia* spp. previously recorded in flat oysters in Tasmania, South Australia, and Western Australia are also *B. exitiosa.*

Marteilia spp. parasites

Marteilia spp. are paramyxean parasites of oysters and the causative agent of the disease marteiliosis. *Marteilia refringens* is an exotic parasite of bivalve molluscs, principally ostreid oysters and mytilid mussels, and causes Aber disease. It is listed as a WOAH-notifiable disease, and is also listed on Australia's National List of Reportable Diseases of Aquatic Animals and on the Exotic Environmental Pest List (EEPL). *Marteilia refringens* is not present in Australia. It is recorded from several countries in the northern hemisphere, including Atlantic Europe such as France and the United Kingdom, and Mediterranean Europe including Croatia, Italy, Spain, and Greece (WOAH 2022). *Marteilia refringens* infects the digestive system of hosts, where it undergoes sporulation, similar to *M. sydneyi* which is present in Australia. Other marine invertebrates are likely involved in the parasite's lifecycle. For example, the copepod, *Paracartia grani,* is suspected to be involved in the transmission of *M. refringens* (Audemard et al. 2004).

Marteilia sydneyi is endemic to Australia and causes QX disease. It infects the native rock oysters (*Saccostrea glomerata* and *S. cucullata*) and has previously been found in New South Wales, Queensland, and Western Australia (Adlard & Nolan 2015).

Bivalve impacts to human health

Bivalves are filter feeders and can accumulate toxins (including heavy metals) and pathogenic microorganisms from the environment through filter feeding activity; this can occur in both native or invasive marine bivalves, or in wild or cultivated bivalves. Pathogens like human norovirus and *Vibrio parahaemolyticus* can cause gastrointestinal infection in humans after consuming infected shellfish. Toxic algae can accumulate in bivalves and cause different types of poisoning in humans, including paralytic shellfish poisoning, which in severe cases can cause human fatalities.

Human health risks are inherent in consumption of native and invasive marine bivalves. Bivalves can accumulate viruses and bacteria present in the water column, including those of concern to human health. For example, levels of faecal indicator bacteria were several-fold higher in *P. perna* mussels than the surrounding water (Boufafa et al. 2021). Collection of bivalves are typically banned after a period of heavy rain to reduce human health risk through ingestion of enteric microorganisms from contaminated stormwater discharge. Appendix A contains taxon-specific information on some invasive marine bivalves, including information on bivalve diseases for each taxon.

Aquaculture

Bivalves constitute an important food source for humans and support important aquaculture production globally. Invasive marine bivalves have a long history of crossing biogeographical boundaries, either accidentally or deliberately, usually for the purpose of establishing aquaculture (Padilla 2010). *Magallana gigas* has been extensively introduced to countries around the world to establish aquaculture and is now the most widely farmed and commercially important bivalve globally (FAO 2018). Fast growth, high fecundity, and environmentally hardy traits that make it a good aquaculture species are less desirable in natural settings where dense aggregations outcompete native species and make the shoreline less inviting for recreational activities (Herbert et al. 2016). In its native range, *P. canaliculus* contributes over half of New Zealand's aquaculture-

source export product (Chaput et al. 2023). *Perna* spp. and *M. bilineata* have also been intentionally introduced throughout the Pacific islands for the purposes of aquaculture (Eldredge 1994). Other genera such as *Ostrea* spp. and *Mytilus* spp. have been introduced for aquaculture purposes in some regions of the world (Michalek et al. 2016).

Overview of invasive marine bivalve management

Management of invasive marine bivalves will depend on the target species, its life history strategy (infaunal vs epifaunal), the size of the incursion (area infested and size of population), and the location. Often the most appropriate management approaches require a combination of several techniques targeting different life stages (see Section 5). The small size of many invasive bivalve species (e.g. *V. gibba* and *M. sallei* have ~30 mm shell lengths), along with the ability for some bivalves to evoke stress-induced spawning when handled, can make physical removal challenging.

Physical removal and biocides can be efficient control methods for small-scale incursions (Hopkins et al. 2011; Willan et al. 2000), but there are no adequate control methods for large-scale marine bivalve incursions. Invasive marine bivalves have been eradicated successfully in Australia (e.g. *M. sallei* in Darwin, NT), however, these incursions have been small and localised (Willan et al. 2000). Although there is the potential for bivalve populations to die out naturally, as was suspected for *P. canaliculus* in South Australia (Richard Willan [MAGNT], pers. comm., April 2023), there is no guarantee that any bivalve incursion will die out from natural causes. Effective biosecurity emergency responses operate under risk-management approaches which assume that each incursion has potential for establishment, spread, and negative impacts. Therefore, any bivalve incursion, especially for high-risk species used as examples within this manual, should be acted on as early as possible during an emergency response.

This response manual provides guidance on managing invasive marine bivalves in Australia. The general principles of management are similar for infaunal and epifaunal bivalve taxa, with timeliness being critical to effective management action. If an introduced bivalve has spawned and larvae have settled over a large area, then control will be far more difficult. For example, *M. japonica* recorded from Tasmania was found to have been present for at least 10 years before it was detected, preventing effective control (Grove et al. 2018). When an incursion cannot be eradicated, it is more realistic to manage the population density and reduce the risk of further spread by human pathways, or by potentially manipulating Allee effects of invasive species (Tobin et al. 2011). Refer to Section 4, Section 5, and Section 6 for further information on methods to detect, control, and dispose of invasive marine bivalves, respectively.

1 Guidance and rationale for incursion response

Every biosecurity incident is unique, as is the response to the incident. Management actions taken during marine pest responses will differ based on variables such as the:

- taxon-specific traits and functional characteristics
- impact significance (environmental, economic, social, or cultural)
- extent and duration of the incursion
- location of the receiving environment and its associated values
- likelihood of eradication
- cost and benefits of control and asset protection.

This section discusses national policies that guide and support marine pest responses by providing a biosecurity response framework, operational guidance, and potential financial arrangements that can be tailored to meet the needs of each unique incident.

1.1 Sources of information

Information on the distribution, ecology, and effects of invasive marine bivalves can be found via a variety of sources, including:

- scientists (including taxonomists and diagnosticians) and technical experts
- primary sources of scientific literature
- online resources on marine pests.

The Marine Pest Sectoral Committee (MPSC) maintains a database of professionals, experts, and research and development (R&D) providers who can provide information on the life history, ecology, and biology of invasive marine bivalves. Contact the MPSC Secretariat for more information: mpsc@aff.gov.au.

Several useful online resources contain summary information on invasive marine bivalves. These include:

- The National Introduced Marine Pest Information System (NIMPIS)
- Marine Pest Response Manuals
- National Priority Pests: Part II Ranking of Australian Marine Pests
- National Control Plans are available for six species, two of which are bivalve species:
  - Asian bag or date mussel (*Arcuatula [Musculista] senhousia*)
  - European basket shell clam (*Varicorbula gibba*)

- Additional species distribution and taxonomic databases can be used to search information on invasive species including:
  - Atlas of Living Australia (ALA)
  - Global Invasive Species Database (GBIF)
  - National Estuarine and Marine Exotic Species Information System (NEMESIS)
  - CABI Compendium
  - World Register of Marine Species (WoRMS)
  - The Australian Taxonomy Community Directory
- Other resources relevant to Australian bivalves include:
  - The Malacological Society of Australasia

1.2 Policies for management of marine pest responses in Australian waters

The Biosecurity Incident Management System (BIMS): Marine pest version manual provides guidance on policies and procedures for the management of biosecurity incident responses, including responses to marine pest emergencies within Australian waters.

1.2.1 Commonwealth, state, and territory authority responsibilities

Lead agencies in a response to a marine pest emergency of an invasive marine bivalve should collaborate with and keep the Consultative Committee on Introduced Marine Pest Emergencies (CCIMPE) informed.

For incidents that are contained to a single jurisdiction, state coordination centres and local control centres may be established depending on the scale of the response. A national coordination centre is established to help manage concurrent incursions in more than one jurisdiction. National coordination operations will work in consultation with CCIMPE representatives and relevant industry and community sector organisations. For further information on local, state, and national control and coordination centres refer to the BIMS: Marine pest version.

1.2.2 Consultative Committee on Introduced Marine Pest Emergencies (CCIMPE)

CCIMPE provides national technical coordination for managing marine pest emergencies and comprises biosecurity representatives from each Australian jurisdiction with coastal borders (the Australian Capital Territory is not represented).

CCIMPE is a national technical body that advises the National Management Group (NMG) on marine pest incidents and whether they meet the criteria for national cost-sharing under the National Environmental Biosecurity Response Agreement 2.0 (NEBRA). The NMG is the peak national biosecurity decision-making forum through which parties seek decisions in the event of an incident of a pest or disease (DAFF 2021). The NEBRA outlines the NMG's role and responsibilities.

CCIMPE provides technical and response advice to lead agencies and assists in developing and implementing response actions such as a National Biosecurity Incident Response Plan (NBIRP).

CCIMPE may also act as an information sharing forum to provide national biosecurity agencies with updates on marine pest responses that are not cost-shared under the NEBRA.

The NEBRA establishes national arrangements for responses to nationally significant biosecurity incidents when they are predominately for environmental or public benefit. The NEBRA provides a mechanism to share responsibilities and costs for a response when eradication is considered feasible, the pest is considered to be of national significance, and the response is calculated to be cost-effective.

1.3 Funding of operations and compensation

The National Environmental Biosecurity Response Agreement 2.0 (NEBRA) establishes national arrangements for responses to nationally significant biosecurity incidents where there are predominately environmental or public benefits. The NEBRA provides a mechanism to share responsibilities and costs for a response when eradication is considered feasible, the pest is considered to be of national significance, and the benefits of a response outweigh the costs and are calculated to be cost-effective as per Schedule 3 of the NEBRA. Guidance on undertaking a benefit-cost analysis (BCA) for marine pest responses is available from Summerson, Hester and Graham (2018). Demonstrating that the benefits of a response outweigh the costs is required when seeking cost-sharing under the NEBRA.

CCIMPE may recommend to the NMG to consider a national cost-shared eradication response under the NEBRA if an incident is considered nationally significant, technically feasible to eradicate, and cost-beneficial to do so. Species on the APMPL and EEPL are already pre-considered to be of national significance.

Cost sharing must be agreed to by the NMG. The eligible costs of emergency eradication responses are shared as follows:

- a 50% share from the Australian Government
- a 50% share collectively from the states and Northern Territory
  - this is calculated for each jurisdiction based on the length of coastline potentially affected by the marine pest as well as their respective human populations
  - only jurisdictions affected or potentially affected by the pest or disease are required to contribute.

The NMG may commit up to $5 million in annual aggregate towards the eligible costs associated with an agreed national biosecurity incident response. If this $5 million is exceeded in any one financial year, the NMG must seek ministerial approval from all parties to continue activities and/or begin new emergency responses. Private beneficiary contributions to a response will be considered by the NMG on a case-by-case basis where there is one or more private beneficiary and no existing arrangements.

Marine pest biosecurity incidents that do not meet the criteria for cost-sharing under the NEBRA will predominately be the responsibility of the lead agencies in the affected jurisdiction undertaking the response, however *ad hoc* resourcing (e.g. financial, human, and physical) may be available through national biosecurity support programs.

Please refer to the current version of the NEBRA or contact the NEBRA custodian nebra@aff.gov.au for more information as the NEBRA may be periodically revised.

1.4 Decision points

Decision points in a biosecurity response may include decisions to stand-down eradication or control operations and transition the response to management, or to declare the pest as absent/eradicated.

Detection of any suspected introduced marine bivalve not known to occur in Australia should initiate an investigation phase. This phase will likely be run concurrently with the initial control actions if initial indications are that the infestation is limited. If the emergency investigation revealed that the incursion was potentially eradicable then the incident manager will prepare a NBIRP and forward to CCIMPE for urgent consideration.

Management of a marine pest emergency of national significance has three phases of activation:

1. investigation and alert phase
2. operations phase
3. stand-down phase.

Further details on decision points can be found in the BIMS: Marine pest version. It is important to note that not all detections of marine pests will initiate a response involving all three phases. For example, the detection of invasive marine bivalves on a vessel may involve a truncated response.

1.4.1 Determining the current status of marine pests

The current status of marine pests (previously called 'proof of freedom[2]') aims to demonstrate to an agreed level of confidence that a pest is at a low enough abundance that it can be regarded as effectively absent, i.e. eradicated in the context of an incursion. This requires a robust and intensive surveillance program during the operations phase of the response. The purpose of determining marine pest status is to inform future decisions, mainly whether a response can be stood down once the associated surveillance is complete, or whether further ongoing management is required. The outcome of surveillance for marine pest status may influence management actions such as movement restrictions, ballast water and biofouling management. See Section 4 for more information on surveillance and delimitation.

The Marine Pest Sectoral Committee (MPSC) has developed national *Policy principles for determining the current status of marine pests* (Appendix B). The policy principles provide stakeholders (governments, industry, and others) with nationally agreed and flexible principles for determining the status (likelihood of presence/absence) of marine pests in defined areas within Australia.

[2] The term 'proof of freedom' was previously used in marine pest responses. However, 'proof of freedom' has different connotations, especially from an agricultural disease perspective. As such, the MPSC agreed to retire usage of 'proof of freedom' for marine pests, and instead have adopted 'current status of marine pests' to describe the evidence that a specific marine pest is absent from a geographical region. 'Proof of freedom' may still be used interchangeably in some circumstances or in older documents.

Responses that are cost-shared under the NEBRA require a 'proof of freedom' phase if eradication is thought to have been achieved. The NEBRA custodian (nebra@aff.gov.au) can provide guidance on developing surveillance programs for marine pest status on request.

Ultimately, surveillance to determine marine pest status will depend upon the context and requirement. CCIMPE can provide advice and connection to expertise to assist in developing a surveillance plan to assess marine pest status during an incursion.

1.5 Health, safety, and environment

1.5.1 Safety of response personnel

The safety of personnel involved in response activities is paramount. Handling certain aquatic animals may be dangerous. Many methods for response activities also involve divers working under water or in outdoor environments. Personnel may work extended hours to achieve control and eradication. Fatigue in personnel can compromise their safety and that of others, particularly if they are working with machinery or in dangerous environments.

1.5.2 Work health and safety during a response

All operations associated with a marine pest incursion must consider relevant Commonwealth, state, and territory government work health and safety (WHS) requirements, standard operating procedures (SOPs) and safety data sheets (SDS) for response activities, including when handling chemicals and samples (e.g. chlorine in liquid form can cause severe burns and is highly toxic if swallowed or inhaled). Operational staff should be appropriately trained in the safe handling and application of dangerous chemicals. Further information on the hazards, safe handling, emergency procedures, and disposal of chemicals is available on the SDS, which should be available to staff working with a chemical.

1.5.3 Environmental considerations

When a response takes place there may be considerable waste generated which requires consideration prior to the commencement of response activities. Certain techniques will generate large quantities of plastic wastes or involve chemical applications, some of which may have residual effects (e.g. cupric compounds). Disposal of large quantities of organic wastes needs careful consideration and appropriate disposal areas and transport corridors identified. When handling or destroying invasive marine bivalves, care must be taken to prevent induced spawning, and all biological material collected and filtered out to avoid reinfesting the marine environment. See Section 6 for more information on disposal.

Response actions may have impacts on non-target species within the response area and an environmental impacts assessment should include non-target species. This may include threatened or listed native species and culturally or economically significant species.

Response actions also need to consider the surrounding environment. Some high priority areas such as reserves, Sea Country, national parks, and Ramsar wetlands will need consideration as to what methods of management are most appropriate. Effective communication regarding public access to locations, including potential restrictions, and when response activities will be completed is crucial.

2 Pathways and vectors of introduction and spread

Introduction pathways for marine pests can be either primary or secondary. A primary pathway moves species to new regions across biogeographic barriers, whereas a secondary pathway is the spread and dispersal of introduced species between points within or between neighbouring regions (Harrower et al. 2018). Once introduced into Australia, marine pests may subsequently spread to new locations by various vectors. Vectors are the physical means, agent, or mechanism that facilitates the transfer of organisms, or their propagules, from one place to another.

Details of pathways and vectors for the introduction and spread of invasive marine bivalves to and throughout Australia are provided in this section. Vectors considered to have the highest risk of introducing invasive marine bivalves to Australian waters are:

- transport of biofouling on seagoing vessels and other maritime infrastructure
- discharge of ballast water.

Invasive marine bivalves can also be introduced and spread by:

- fisheries, aquaculture, and the ornamental trade (deliberate or accidental)
- transport via recreational vessels and bilge water (Fletcher et al. 2017; Fletcher et al. 2021)
- natural dispersal (e.g. ocean currents)
- debris and flotsam.

Table 2 presents a summary of known pathways and vectors for introduction and translocation of invasive marine bivalves to Australia. These same pathways and vectors for introductions are likely modes of transport for other bivalve species not included in this manual.

Bivalves can be transported over large distances and introduced into new areas as larval stages or as adults. It is often unclear what the specific vector is for introduction unless the bivalve was directly observed being introduced by the vector. Biofouling is a common pathway for introduction and bivalves are often observed attached to hull surfaces and niche areas during vessel inspections or other underwater activities.

The most common vectors for transporting invasive marine bivalves are biofouling and ballast water associated with vessel movements. Vessels that may be at port for prolonged periods are susceptible to fouling organisms, and bivalves such as *Perna* spp. are frequently found on vessel hulls and niches. Both commercial and recreational vessels can transport invasive marine bivalves.

Bivalves are an important food source and form substantial aquaculture industries globally. There are numerous historical reports of intentional introductions of invasive marine bivalves for the purposes of establishing aquaculture for human consumption. Today, deliberate introductions of bivalves to establish aquaculture is unlikely to be an important pathway into Australia because of strict import requirements for bivalves and bivalve products. However, for invasive marine bivalves

already established in Australia, deliberate movements of these bivalves for fisheries or aquaculture purposes may cause secondary spread.

Other pathways include natural dispersal of larvae in ocean currents or passive dispersal via fouled debris and flotsam. These vectors are less likely to introduce marine bivalves into Australia because Australia is considered to be geographically isolated, restricting natural dispersal events. However, parts of northern Australia are susceptible to marine debris from southeast Asia (Wilcox et al. 2015), which can have fouling bivalves attached (Póvoa et al. 2021). Natural dispersal and marine debris can facilitate secondary spread at localised scales (i.e. within an embayment, estuary, or marina). Recreational vessels travelling between marinas at localised scales may also be a source of secondary spread.

Once introduced into Australia via a primary pathway, invasive marine bivalves may subsequently spread to new locations within Australia by the same vectors that introduced them, or another secondary pathway. DNA sequencing of invasive bivalves can enable the provenance or potential source location to be more easily identified (Dias et al. 2018).

Some bivalves have life history strategies that make them more likely to spread including long-lived larval stages, broad environmental tolerances, occupation of shallow water habitats where they are more likely to encounter vessels, or by being part of a fouling community that colonises vessels. Invasive marine bivalves such as *M. sallei*, *Perna* spp., *M. strigata, P. amurensis* and *M. japonica* all possess biological traits that increase the likelihood of successful invasions.

Details of pathways and vectors for the introduction and spread of invasive marine bivalves to and throughout Australia are provided below. Notably, this section covers the different mechanisms underpinning these vectors. Further information on managing these vectors and associated policies and guidelines are found in Section 3.3.

Table 2 Summary of the pathways and vectors which transport invasive marine bivalve species into and within Australia

Pathway	Vector description	*Arcuatula senhousia*	*Magallana* spp.[a]	*Mya* spp.[b]	*Mytella strigata*	*Mytilopsis sallei*	*Perna* spp.[c]	*Potamocorbula amurensis*	*Varicorbula gibba*
Vessels	Biofouling (including niche areas)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Vessels	Ballast	Yes	Yes	Yes	Yes	Yes[d]	Yes	Yes	Yes
Fisheries and aquaculture	Accidental translocation with aquaculture stock movement	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Fisheries and aquaculture	Accidental translocation with fishing products, for example bait	Yes	Yes	Yes	No	Yes	No	No	No
Fisheries and aquaculture	Illegal intentional introduction	No	Yes	No	No	No	Yes	No	No
Natural dispersal	Natural range extension through larvae	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Debris and flotsam	Dispersal associated with debris and flotsam	Yes	Yes	No	Yes	Yes	Yes	No	No

a Pathways and vectors will be similar for *Magallana ariakensis, M. bilineata,* and *M. gigas*

b Pathways and vectors will be similar for *Mya arenaria* and *M. japonica*.

c Pathways and vectors will be similar for *Perna canaliculus, P. perna* and *P. viridis*

d Unlikely over long distances because of the short larval period (approximately 2 days).

2.1 Biofouling

Biofouling can occur on all fixed or mobile structures immersed or exposed to the water. Fouling communities typically comprise sessile and encrusting organisms such as algae, barnacles, bivalves, tubeworms, hydroids, and ascidians that have attached and are in a sessile life-stage. If the fouling layer is dense enough, it can provide shelter and support mobile species such as amphipods, crabs, seastars, and fish that may live in or among the fouling species.

International and domestic shipping has facilitated the spread of marine pests more than any other vector due to transport in ballast water and biofouling assemblages (Hewitt et al. 2009). Potential vectors include a diverse range of craft, including commercial vessels, such as tankers and container vessels, military vessels, fishing vessels, recreational vessels, passenger vessels, barges, dredges, and research vessels. Biofouling on the vessel hull or internal seawater systems are the main ways that vessels can act as vectors for invasive marine bivalves.

Species within biofouling assemblages can be introduced by:

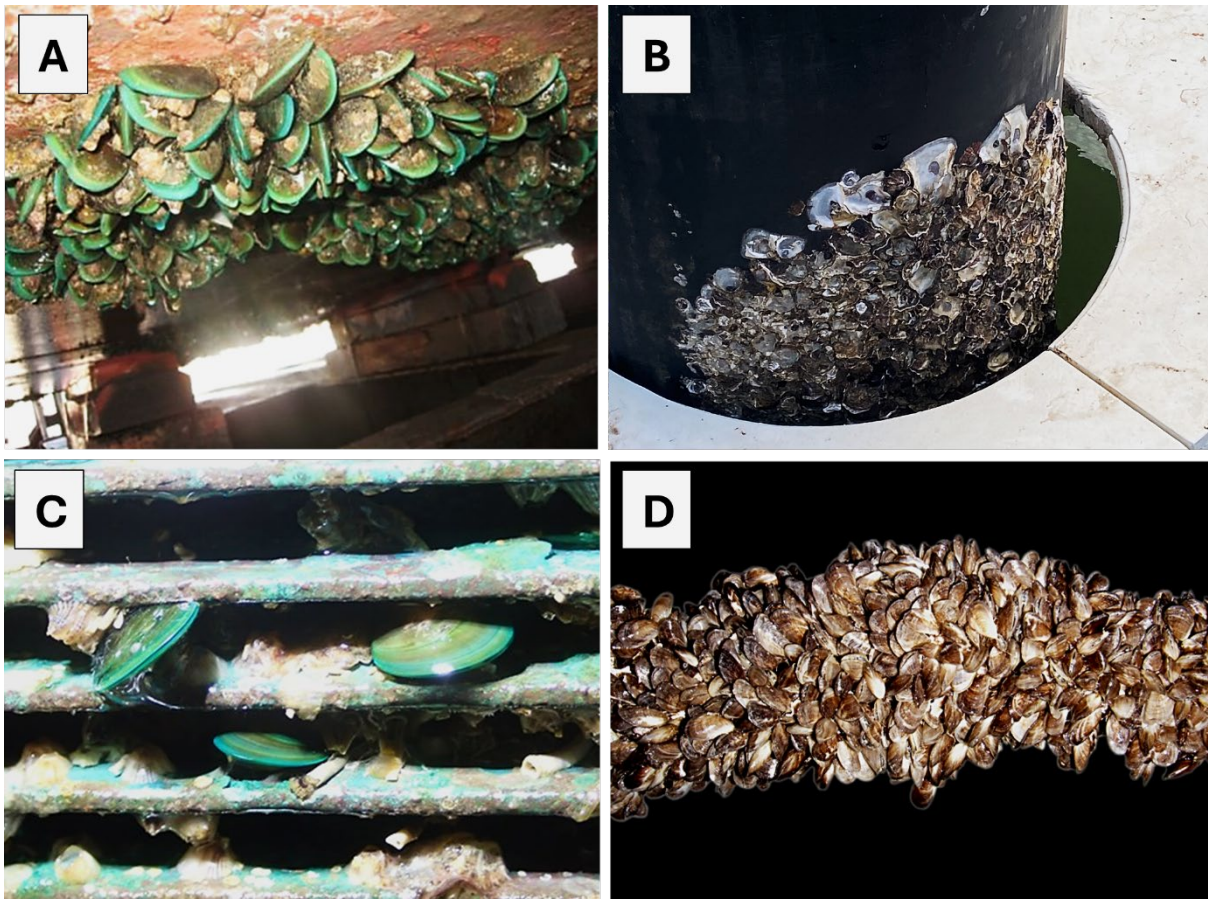
- spawning or fragmentation of a fouling species present on a vessel while in port followed by its successful settlement and establishment of a reproductive population
- the dislodgment or disturbance of fouling species from a vessel in port (e.g. through hull cleaning or abrasion with wharf piles)
- the sinking of a fouled vessel (MPSC 2021).

Vessel niche areas may be more susceptible to biofouling attachment and growth due to different hydrodynamic forces, susceptibility to coating system wear or damage, or not being adequately painted with anti-fouling coatings. These areas include, but are not limited to, sea chests, bow thrusters, propeller shafts and guards, inlet gratings, and dry-dock support strips.

Niche areas make up only a small portion of a vessel's surface, yet because niche areas are more likely to be fouled, they constitute a high biosecurity risk. As niche area fouling is less likely to affect hydrodynamics and fuel consumption there is usually less incentive to ensure these areas are kept clean. Cleaning niche areas can be time consuming because of the need to take off external grates or covers for access. However, sea chests are integral for engine cooling, ballast management, and fire-prevention systems, so it is vital for sea chests to be kept clear of biofouling to allow these systems to operate properly.

Epifaunal bivalves are common biofouling species because of their ability to settle on a variety of hard substrata and in large numbers. Bivalves have been documented from vessel biofouling in Australia and have also been seen growing on marine infrastructure in the environment (Figure 3). After the successful eradication of *M. sallei* from Darwin in 1999, divers undertaking monitoring surveys for *M. sallei* continued to discover infestations on vessels moored in Australian waters (Willan et al. 2000). Epifaunal bivalves *P. viridis* and *P. canaliculus* have been detected multiple times on vessels in Australia. *Perna viridis* has been found in the sea chest water intake areas, strainer baskets, and propellor areas as well as attached to the hull of vessels visiting Australia (Heersink et al. 2020; Wells 2017). Fouling organisms such as barnacles and oysters can provide substrate for attachment. Epifaunal bivalves can also grow on marine infrastructure that is submerged for long periods of time, such as ropes and jetty pylons.

Figure 3 Examples of biofouling on different marine structures by invasive marine bivalves



A Biofouling of *Perna viridis* under a vessel in dry-dock. **B** Biofouling of *Magallana bilineata* on a pontoon. **C**. Biofouling of *P. viridis* on a vessel niche area (sacrificial anode). **D** Biofouling of *Mytilopsis sallei* on a rope.

Source: (A) Queensland Government; (B) Evan Rees, NAQS; (C) Darwin Dive Company; (D) MAGNT Collection

Marine infaunal bivalves are unlikely to be fouling species because of their life habit. For example, *M. japonica* is a deep burrowing clam and is therefore less likely to be spread via biofouling and more likely to be transported with ballast water or ballast tank sediments. However, infaunal species have been reported from biofouling communities of vessels. For example, approximately 50 *V. gibba* were found amongst the mud in the bottom of a sea chest of a passenger ferry in southeast Australia (Coutts et al. 2003). Juvenile *P. amurensis* were recorded on the hulls of obsolete vessels (Davidson et al. 2008). The presence of juvenile clams on the vessel hulls likely resulted from the colonisation of bryozoan mats and associated sediment accumulation. Sediment associated with biofouling needs to be considered as an important pathway for infaunal bivalves.

Dry-docking and in-water operation principles and recommendations are contained in the Anti-fouling and in-water cleaning guidelines. The guidelines provide guidance on best-practice approaches for the application, maintenance, removal, and disposal of anti-fouling coatings and the management of biofouling and invasive aquatic species on vessels and movable structures in Australia and New Zealand. The practices described in these guidelines have been aligned with international conventions intended to protect the aquatic environment from invasive aquatic species and contaminants from shipping. These include the:

- International convention on the control of harmful anti-fouling systems on vessels
- The 1996 protocol to the Convention on the prevention of marine pollution by dumping of wastes and other matter, 1972
- The 2011 Guidelines for the control and management of a ships' biofouling to minimize the transfer of invasive aquatic species.
  - The 2011 guidelines have been updated and an exposure draft of the revised guidelines is available on the Department of Agriculture, Fisheries and Forestry (DAFF) website at: In-water cleaning in Australia - DAFF (agriculture.gov.au). The revised guidelines are undergoing consultation, and finalised guidelines will be published accordingly.

The *Biosecurity Act 2015* can be used in the absence of appropriate state or territory legislative powers and may be used in circumstances that include directing conveyances.

Marine aquaculture equipment such as buoys, ropes, nets, and cages can contribute to the spread of marine pests if they are fouled. During a marine pest emergency response, the cleaning of stock and equipment, and reduced or ceased movement of these items should be appropriately managed.

Fixed marine structures such as pontoons, moorings, or piles do not represent a risk for translocation of marine pests unless they are moved while still fouled. If an emergency response to a marine pest is underway, then scheduled installation or repair of marine structures should be appropriately managed, including any support vessels or equipment used.

Biofouling management for vessels and infrastructure should be consistent with the National Biofouling Management Guidelines. These are available for the following industries and operators:

- aquaculture industry
- offshore infrastructure (petroleum production and exploration industry)
- port and marina operators (marinas, slipways, boat maintenance and recreational boating facilities)
- vessels:
  - commercial fishing vessel
  - commercial vessel
  - non-trading vessel
  - recreational vessel.

Further information on managing vessel biofouling and associated policies and guidelines are detailed in Section 3.3.1.

2.2 Ballast

Ballast water is water taken on-board by vessels to maintain stability and trim. Ballast water is used by most modern-day vessels, notably large commercial vessels, some cruise vessels, and certain types of fishing vessels, yachts, and ferries. Unladen vessels arriving in a port will usually be ballasted and will need to discharge some ballast water in proportion to the weight increase caused by cargo loading. The number and frequency of species introductions has increased since ballast water

replaced solid ballast around the 1880s (Carlton & Geller 1993). Around 20% of introduced marine species into Port Phillip Bay, Victoria, are estimated to have arrived in ballast water (Hewitt et al. 2004).

Ballast water is a relatively non-selective dispersal mechanism that can carry bivalve species as planktonic stages (e.g. gametes or larvae), free swimming juveniles or adults, and fouling bivalves attached to the vertical walls of the ballast compartments (Carlton 1985; Davidson & Simkanin 2012). Sediments can also be inadvertently taken up along with the ballast water and can accumulate in the ballast tank, providing habitat for benthic organisms including infaunal bivalves that may be transported to other locations (Carlton 1985; Davidson & Simkanin 2012).

Bivalve species with relatively long larval durations are more likely to survive within ballast water than bivalves with shorter larval stages. For example, *P. canaliculus* with a larval duration of 3 to 6 weeks (Jeffs et al. 1999) is more likely to be introduced via ballast than *M. sallei* which has a larval duration of around two days (Kalyanasundaram 1975).

Wide environmental tolerances will influence a species' ability to successfully transfer via ballast. *Potamocorbula amurensis* is likely to have been introduced to San Francisco, USA, by ballast water because of its long larval duration and ability to tolerate large changes in salinity (Nicolini & Penry 2000).

The *International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004* (the BWM Convention) was adopted to manage the risks associated with the transfer of organisms via ballast water. Australia is a signatory to the BWM Convention which entered into force 8 September 2017. Chapter 5 of the *Biosecurity Act 2015* is dedicated to the management of the biosecurity risks associated with ballast water and ballast tank sediments in Australian seas.

The Australian Ballast Water Management Requirements (the ABWM Requirements) provide information and direction on the obligations of vessel operators with regards to the management of ballast water and ballast tank sediments in Australian seas. The ABWM Requirements apply to all vessels operating internationally and domestically in Australia.

From 8 September 2024, all vessels constructed after 1 January 2009 that are subject to regulation B-3 of the Annex to the BWM Convention must adhere to the performance standards contained in regulation D-2 of the BWM Convention. Regulation D-2 of the Annex to the BWM Convention sets the biological performance standards in discharged ballast water and contains maximum numbers of organisms across different size classes that represent planktonic organisms and human health-associated microbes. For discharged ballast water to comply with the Regulation D-2 standard, most vessels will have installed an International Maritime Organization (IMO) Type Approved ballast water management system (BWMS).

Further information on managing vessel ballast water and associated policies and guidelines are detailed in Section 3.3.2.

2.3 Fisheries, aquaculture, and the ornamental trade

Fishing and aquaculture operations and the ornamental trade can translocate bivalves accidentally with other stock or bait movements, or deliberately by illegal movements of live bivalves for the purpose of establishing a population for cultivation. Bivalves may also be accidently transported on aquaculture and fisheries equipment such as buoys, ropes, nets, and cages. Although there are strict regulations of live animal imports, there is still some risk of introduction of an invasive marine bivalve into Australia via importing aquaculture or fisheries stock. Imported aquaculture stock already processed for human consumption is non-viable and import of used aquaculture equipment is closely managed.

The fisheries and aquaculture trade has historically been an important pathway for introduction of marine bivalves into Australia and around the world, particularly for commercially important species such as *M. gigas* and *Perna* spp. Bivalves are highly valuable as a human food item and *M. gigas* dominates global aquaculture production and is an economically and socially important aquaculture species in Australia. *Magallana gigas* was intentionally introduced into southern Tasmania, Western Australia, and Victoria in the mid-1900s. *Magallana gigas* was later found in New South Wales in the 1960s, where it was believed to have been illegally intentionally introduced (NSW DPIRD 2024). Import of aquaculture stock into Australia is strongly regulated, reducing the biosecurity risk from this vector.

There is risk of marine pest translocation within Australia through domestic trade of live aquatic animals for socio-economic and environmental benefit (DAFF 2020a). For instance, due to the species' economic importance in New Zealand, if *P. canaliculus* were to establish in Australia, illegal anthropogenic domestic spread would need to be managed. The National policy guidelines for the translocation of live aquatic animals have been developed to guide any translocation activity of live aquatic animals.

The ornamental trade is not as significant for the translocation of invasive marine bivalves compared to fisheries and aquaculture. The sale of 'live rocks' are common among the ornamental aquarium trade. Live rocks are taken directly from the ocean, often inhabited by a multitude of marine organisms, including bivalves, that have been introduced into an aquarium. Live rock is sold in Australia with Queensland, Western Australia, Northern Territory and Victoria common places of origin (Morrissey et al. 2011). Although internet sales data shows that live rock sales are mainly collected and sold within jurisdictions, it is a potentially important vector, particularly for domestic spread of bivalves that inhabit rocks and other complex structures. Gravel and aquarium water released into waterways may also spread any bivalves (larvae or other life stages) present. Several species of clams such as *Tridacna* spp. and *Hippopus* spp. are also available to aquarium hobbyists, which can be shipped domestically outside of their native ranges. The importation of live rock with viable invertebrates and import of viable bivalves is banned (see Australian Biosecurity Import Conditions - BICON).

Further information on managing aquaculture stock and equipment and associated policies and guidelines are detailed in Section 3.3.3.

2.4 Natural dispersal

Natural dispersal is a mechanism for the range expansion of a species through the movement of gametes, larvae, or adults to a new location via natural mechanisms in the environment, such as wind or ocean currents. Characteristics that enable invasive marine bivalves to be spread via this pathway include having a planktonic dispersal phase or ability to foul floating objects.

Although anthropogenic vectors are the most common mechanism for transporting bivalves over long distances (i.e. international and national scales), once a bivalve has been introduced into an area, it can disperse naturally over shorter distances at local and regional scales. Control of natural dispersal from established populations is likely to be impractical or impossible, which is why response actions need to be taken before a population can establish.

Bivalves are primarily broadcast spawners with a single female bivalve capable of releasing tens of thousands of eggs into the environment during one spawning event. Planktonic larvae are capable of being dispersed widely via currents (Dias et al. 2018). Several bivalves have continually spread from a focal point after their introduction. The spread of *P. amurensis* throughout San Francisco Bay is thought to be from natural dispersal via juvenile clams drifting with the tide. A common characteristic of *M. gigas* introductions is that this species can go from a relatively confined aquaculture population to becoming a major biomass component of wild systems via natural dispersal.

2.5 Debris and flotsam

Although introductions of bivalves via debris and flotsam are rare, it can be an important pathway under certain circumstances, such as following a natural disaster or shipwrecks. The propensity of bivalve molluscs to foul structures means they can attach themselves to debris and flotsam and drift with ocean currents (Póvoa et al. 2021). Debris can be carried over long distances. Debris from the 2011 Japanese earthquake and tsunami drifted by ocean currents across the Pacific and washed-up on the west coast of North America, bringing with it a diverse range of introduced species, including the bivalve *Mytilus galloprovincialis* (Therriault et al. 2018). In Australia, *P. viridis* was reported from a log washed up on the beach of Mornington Island in 2019. Marine debris from southeast Asia has also been found washed ashore in northern Australia (Wilcox et al. 2015). Debris may also be the cause of important secondary spread within a species' introduced range, especially after storms or flooding events on coastlines.

3 Preventing and monitoring spread

The likelihood for eradication of an incursion by an invasive marine bivalve will increase with early detection and rapid action. Eradication is most likely to be successful in shallow and/or partially or fully enclosed waterways where the incursion can be contained and where natural dispersal is limited. In open coastal waters with moderate-to-high water exchange, emergency containment is likely to be restricted to those bivalves with limited adult and larval dispersal. Management to prevent or minimise further spread or reduce populations may be more appropriate where surveys indicate that an incursion is widespread. In all cases, intensive public consultation and education is essential to ensure support and/or compliance with response actions.

This section covers the basis of invasive marine bivalve containment or eradication from the infested area and any potentially contaminated vectors by explanation of principles for preventing and monitoring spread, including:

- vector management to prevent spread
- surveillance of high-risk vectors
- management of infected vectors and marine infrastructure
- tracing the incursion.

3.1 Management to prevent spread

Preventing the spread of the invasive marine bivalve may include the following management practices, which are best implemented early in the response:

- public communication and engagement
- quarantine and movement controls
- delimitation
- containment where possible
- collection and disposal of small infestations before spawning.

These management practices may also be applicable at any stage of the following response phases:

- investigation phase and alert phase
- operations phase
- stand-down phase.

3.1.1 Public communication and engagement

Sometimes referred to as public relations, this is the management and communication of public information and perceptions. Communication and engagement with all stakeholders, including Commonwealth, state, and territory government agencies, industry, and community partners are critical to gain acceptance of management or eradication attempts, compliance with any regulations, and to encourage participation in surveillance activities and reporting.

Communication and engagement should occur early in any invasive marine bivalve response and should be maintained during recovery efforts and until the end of the stand-down phase.

The affected jurisdiction may establish an Incident Management Team (IMT), in which a Public Information function will be activated. The Public Information function covers the overall strategic communication approach to the incident including specific activities: call centre operation, media, social media, website content, community and stakeholder engagement, as well as the development of collateral such as flyers, signage, and similar communication materials.

The Public Information function works with the National Biosecurity Communication and Engagement Network (NBCEN) to develop nationally consistent messaging. Regardless of incident level, the NBCEN can be used to coordinate the public information response nationally (Animal Health Australia 2023) The NBCEN consists of a communication representative from each jurisdiction including other relevant organisations which can provide technical expertise. A member from NBCEN (usually the Commonwealth representative) attends the Consultative Committee on Introduced Marine Pest Emergencies (CCIMPE) meetings and develops national talking points in conjunction with the combat jurisdiction to facilitate the delivery of consistent messaging that can be agreed to and used by all jurisdictions. The NBCEN is guided by the Biosecurity Incident Public Information Manual (BIPIM). More on the national arrangements, including NBCEN, can be found on the Outbreak website.

Public communication and engagement need to consider affected individuals and businesses and the economic and social (e.g. mental health) aspects of impacts of response activities. Relief and recovery support may need to be coordinated for emergency-affected individuals and communities. The BIMS: Marine pest version provides guidance on relief and recovery roles in a biosecurity response context.

3.1.2 Quarantine and movement controls

Quarantine and movement controls can be implemented during the investigation phase, alert phase, and operations phase, and are best implemented early (where possible) and refined when investigative work has provided additional information. These measures may end up being permanently implemented to minimise risk of spread or impacts in a long-term management program.

When a suspected invasive marine bivalve is detected in an area, but a marine pest emergency has not yet been confirmed, the combat jurisdiction (notifying party) should take steps to limit the spread of the suspected pest from the investigation site or area. Limiting spread can be assisted by initiating restrictions on movements of potential vectors or release of water where this may contain propagules. Researchers and response field staff have a vital role to play in management of spread, including visibly implementing rigorous biosecurity measures when moving around the area.

3.1.3 Delimitation

A delimiting survey establishes the geographic extent of an area considered to be infested by an invasive marine bivalve and will also identify areas where the bivalve is deemed to be absent. As part of the investigation phase, delimitation informs feasibility of eradication and areas to target for eradication or control and management. Delimitation may also occur throughout the later phases of the response to inform the next steps of a response or determining the status of a marine pest

(present or absent). In some cases, delimitation may take over one year to capture the seasonal appearance of some invasive marine bivalves.

For more information on delimiting an incursion, see Section 4.1.

3.1.4 Tracing an incursion

Tracing is used to discover the mechanism and pattern of the spread of an invasive marine bivalve and may include trace-forward and trace-back. Tracing back is used to discover where an incursion may have originated from and identify additional outbreak sites within Australia. The first location to have the detection of an invasive marine bivalve may not be the original site of introduction. Tracing is crucial to defining and modifying the dimensions of specified areas defined in Figure 4.

Tracing an incursion usually occurs at the same time as a delimiting survey (refer to Section 4.1). Trace-back and trace-forward information is used to determine how and where a marine pest first entered a site and where it may possibly spread to (van Havre & Whittle 2015).

Tracing an incursion requires investigations into:

- the length of time the species has been present
- the initial source and location of infestation
- whether the pest is likely to have reproduced
- the possible movement of water, vessels, animals, submersible equipment, and other potential vectors for the pest to and from the site
- the existence and location of other potentially infested areas, particularly areas of suitable habitat.

3.1.4.1 Trace-back

Trace-back information can be used to determine the possible extent of an incursion, particularly for a primary incursion where a single size or age class is present. Working backwards from the estimated age of the specimens and the known settlement biology and larval lifecycle of the species, hydrodynamic modelling can estimate the source of a spawning event. This source information can be used to determine where else in the area the prevailing currents could have spread the larvae (Burgman et al. 2013; Hauser et al. 2016). The use of DNA-based methods can help identify both source and connected populations and areas of provenance (Roux et al. 2020).

Elements of demography of the invasive marine bivalve populations may be inferred from the size or age distribution within the population and reproductive state of bivalves collected during investigations. A population that contains individuals that vary widely in size, are reproductively active, or contain two or more distinct size cohorts, could be indicative of successful local reproduction and multiple recruitment events.

3.1.4.2 Trace-forward

Trace-forward information can be used to identify locations outside the infested area that may have been exposed to the pests by vectors that have departed the known infested area (van Havre & Whittle 2015). Areas near detection sites can be surveyed in more detail on pest distribution or abundance if needed for assessment of eradication feasibility. Surveillance of areas of potential

secondary spread can then be prioritised based on risk, informed by vectors, modelling, and habitat suitability (Brown et al. 2013).

For more information on data sources for tracing vectors, see Section 4.1.1.2.

3.1.5 Investigation and alert phase

3.1.5.1 Investigation phase

The investigation phase includes confirmation of the bivalve's species identity and should attempt to identify all potential vectors present at the outbreak site. Species identification is confirmed using morphological features via taxonomic experts, molecular diagnostics such as qPCR and DNA sequencing, or a combination of traditional taxonomy and molecular methods (see Section 4.3.1).

Concurrent management actions need to be undertaken while species identification is being confirmed. If necessary, where morphological identification will take some time, molecular identification may be sufficient to act on. The combat jurisdiction should notify the CCIMPE Secretariat (CCIMPE@aff.gov.au) of the suspect incursion within 24 hours via email, which permits eligibility for NEBRA consideration. This is classed as an informal notification. The Australian Chief Environmental Biosecurity Officer (ACEBO) will also be informally notified of the suspect detection via the CCIMPE Secretariat.

Once confirmation is received on the species identity, the combat jurisdiction should submit a Preliminary Information Data Sheet (PIDS) containing details on the initial detection to the CCIMPE Secretariat via email. The submission of the PIDS is the formal notification of the detection. The AECBO is also formally notified of the confirmed detection via the CCIMPE Secretariat, and the PIDS is circulated to CCIMPE including any actions to be taken. The combat jurisdiction may request CCIMPE to convene a meeting to provide technical advice on the incident.

Potential vectors for invasive marine bivalves are discussed in Section 2. As a first step in the investigation phase, relevant parties should be notified about the investigation into a marine pest incident in the relevant area (e.g. port authorities, marina operators, vessel owners, and aquaculture facilities). Cooperation from stakeholders is important in order to stop, restrict, or inform the combat jurisdiction of the risks associated with movement of vectors to and from the site. Compliance with movement controls may be enhanced by communication and distribution of appropriate public awareness materials about the pest.

Care needs to be taken when transporting specimens to avoid any chance of accidental release. In this phase, appropriate local authorities need to be contacted to obtain permission for relevant surveillance and sampling activities in specified areas (e.g. marine parks, conservation areas, and nature reserves), and for dealing with species listed in relevant legislation of any state or territory waters. Please refer to Appendix E in the Marine pest response manual for suitable specimen-handling techniques when sampling bivalves. That appendix has sample handling and preferred narcotising, fixation, and preservation techniques for bivalves. It also gives advice on appropriate levels of experience required for sample processing.

3.1.5.2 Alert phase

If the initial investigation finds that an invasive marine bivalve is likely to be present, the combat jurisdiction should communicate the findings to CCIMPE for consideration of the appropriate course of action recommended by the affected jurisdiction to manage the risk of spread from affected sites.

During the alert phase, an incident management team (IMT) may be appointed to confirm the identification of the suspected invasive marine bivalve and the likely extent of an incursion. The IMT is established by the incident manager and works from its designated operations centre. Staff performing incident management functions should have the appropriate skills, knowledge, and experience to perform incident management functions, where possible. For further information on the functions of an IMT, see BIMS: Marine pest version.

The IMT must ensure appropriate measures are implemented. These could include:

- restrictions on movement of potential vectors, such as vessels, fishing gear, and aquaculture equipment into and out of suspect areas
- managing the movement of people, such as property owners, business owners and employees, tourists, scientists, into or out of suspect areas, as appropriate. This may require police involvement
- managing water movements where possible
- promoting awareness of methods to report sightings of the pest and access general information
- tracing potential vectors that have left the affected site
- hydrodynamic modelling to determine potential spread of larval stages or gametes of bivalves
- redirecting vessels that have already left the site to appropriate sites for inspection and/or decontamination if appropriate
- informing other destination jurisdictions of vessel movements from the high-risk areas
- notifying relevant experts when appropriate.

If required during the alert phase and following CCIMPE endorsement, a National Biosecurity Incident Response Plan (NBIRP) may be submitted to the National Management Group (NMG) for consideration of national cost-sharing arrangements under the National Environmental Biosecurity Response Agreement 2.0 (NEBRA) to help resource a national biosecurity incident response. In such instances, the NMG makes decisions that inform the national coordination of the response, while CCIMPE provides the technical advice on measures required.

3.1.6 Operations phase

The operations phase will be guided by whether eradication of the invasive marine bivalve is determined to be feasible or not feasible. An assessment is undertaken in accordance with Schedule 3 of the NEBRA to determine the technical feasibility of eradicating the invasive bivalve during a proposed national response. The feasibility of undertaking a national response is based on conclusions reached by using scientific information to evaluate the proposed response.

For more information, see the Schedule 3 of the NEBRA.

3.1.6.1 Eradication considered feasible

If an investigation reveals a potentially eradicable incursion of an invasive marine bivalve, then movement restrictions implemented in the investigation phase should remain in place and amended as appropriate to reflect emerging information.

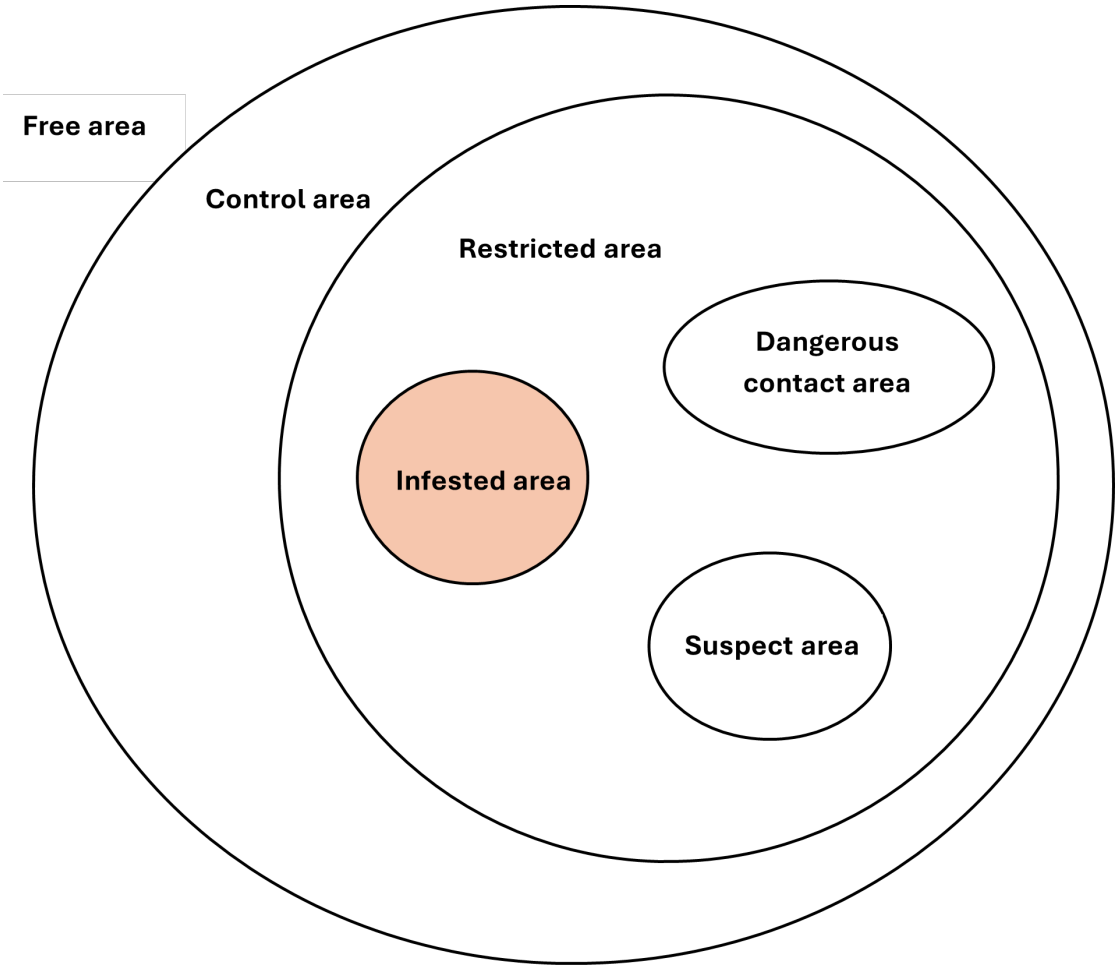
Quarantine restrictions require establishing specified areas (Figure 4):

- Infested area – all or part of a waterway in which a marine pest incident is known or deemed to exist, pending confirmation of pest identification
- Dangerous contact area(s) – an area close to an infested area in which a marine pest has not been detected but due to its potential for infestation, will be subject to the same movement restrictions as an infested area
- Suspect area – an 'at-risk' area which may be linked to the infested area or has the potential to harbour a marine pest and is subject to the same movement restrictions as an infested area, pending further investigation
- Restricted area – surrounds an infested area, dangerous contact area, and suspect area and is subject to intensive surveillance and movement controls of potential vectors[3]
- Control area – surrounds the restricted area in which biosecurity conditions apply to the entry or exit of potential vectors or specific risk items.

Similar terminology is applied to potentially infested vectors within each area. For example, a vessel within a dangerous contact area would be classified as a 'dangerous contact vessel' and a vessel within an infested area would be classified as an 'infested vessel'. For more information on response area classifications, see the BIMS: Marine Pest Version.

[3] The legislative ability and scope of powers to establish biosecurity restricted areas and control areas will depend on the biosecurity legislation in the relevant jurisdiction.

Figure 4 Specified areas that may be designated during a marine pest emergency



Source: Adapted from BIMS: Marine pest version (2020)

The extent of each specified area should be determined by:

- an initial delimiting survey of the area (see Section 4.1.1 for guidelines on designing a delimiting survey)
- an evaluation of the length of time the species has been present and whether it is likely to have reproduced. This could be calculated by the size and distribution of the bivalves in the affected area, the number of cohorts apparent and, when possible, examination of the reproductive status (e.g. evidence of mature gonads – see McDonald et al. 2018)
- larval period and dispersal capability
- the strength and distribution of directional or tidal currents, or other episodic weather events
- expert advice.

It is important to recognise that in aquatic situations a simple radius around a detection is inadequate. Hydrodynamics, physical or chemical parameters of the habitat, geography of the area, and ecology of the target species need to be considered to determine the specified areas.

Movement restrictions may include limiting:

- the movement of vessels
- equipment exposed to the pest (will vary depending on target species)
- aquaculture stock or equipment
- access to and within certain areas
- the uptake or movement of ballast water or other water (such as influent and effluent water from land-based aquaculture or managed water bodies) within the control areas where appropriate controls are not in place.

Implementation of restrictions will be a dynamic process, determined by the location and extent of the infestation and whether the aim is to eradicate the pest or to control its spread. Some restrictions may be deemed impractical or unnecessary in a circumstance, but others will be critically important for eradication or control. Effective communication and accurate information dissemination are critical to ensure compliance and acceptance of restrictions.

For more information on incident management functions, see the BIMS: Marine pest version.

3.1.6.2 Eradication considered not feasible

If an investigation reveals an incursion of an invasive marine bivalve is unlikely to be eradicable, then interim containment measures to prevent translocation from any infested waterway should be implemented to minimise the risk of the pest being spread from the affected area.

If CCIMPE determines that eradication is not feasible, CCIMPE will provide this recommendation and formal advice to the NMG. The NMG will make a final decision on this recommendation and whether to move into a stand-down phase. A stand-down phase may be entered either directly from the alert phase or from the operations phase when NMG agrees with CCIMPE's recommendation that there is no need to initiate a national biosecurity incident response.

The stand-down of the NMG does not mean that actions and consultation within CCIMPE cease. This consultation and communication through CCIMPE will continue as long as the affected jurisdiction(s) and/or CCIMPE deem it necessary. Agreement for longer term management and resourcing options should be formulated and agreed to, and resourcing for longer term management determined. Although a stand-down phase may be entered, jurisdictions may transition from an operational phase to management.

3.1.6.3 The Australian Government *Biosecurity Act 2015*

The *Biosecurity Act 2015* can be used in the absence of appropriate state or territory legislative powers and may be used in certain circumstances, including directing conveyances[4] (Appendix C):

- into port
- to not enter a port and to obey further instruction
- to undergo a treatment action deemed necessary by the incident manager.

The Australian Director of Biosecurity (or their delegate) can authorise state and territory officers as biosecurity officers under the *Biosecurity Act 2015*, which will enable certain actions to be

[4] Under the *Biosecurity Act 2015*, the definition of conveyances includes vessels and floating structures.

undertaken in a biosecurity response. All actions taken against a conveyance should only be taken in relation to those identified as being at risk of spreading the invasive species (Ferguson 2000). The *Biosecurity Act 2015* is only intended to be used if there is no state or territory legislation that provides appropriate powers necessary for the response, aside from ballast water which is entirely covered by the *Biosecurity Act 2015*. A provisional list of other Commonwealth, state, and territory powers for intervention and detention of vessels is in Appendix D.

State and territories should consider enacting relevant fisheries or other legislation to prevent or control fishing within a control area and prevent or control translocation of stock and equipment from within it. Any requested movement of fishing gear or aquaculture stock or equipment should be subject to risk assessment consistent with procedures outlined in the National Policy Guidelines for the Translocation of Live Aquatic Animals (DAFF 2020a). All potentially infested fishing gear, aquaculture equipment, or stock should be treated and inspected before removal from the control area.

Refer to Section 3.3.1 on vessel biofouling management and Section 3.3.2 on ballast water management relevant information.

For additional information on using the *Biosecurity Act 2015* during an emergency response see Appendix C.

3.1.7 Stand-down phase

The stand-down phase is in effect when, following appropriate consultation between the affected jurisdiction(s) and CCIMPE, all agree that there is no need to progress or continue with a national biosecurity incident response. During the stand-down phase:

- a systematic approach to winding down operations must be taken to ensure operational effectiveness is not jeopardised
- all personnel, agencies, and industry contacts involved in the emergency response are to be notified of the stand-down
- where the pest is not eradicable, alternative ongoing management options are to be considered and the most appropriate option implemented, given the risk and required investment
- transition to management and recovery options are investigated prior to and throughout stand-down
- the outcomes of the response, and information on the management of the species going forward, should be communicated to stakeholders
- a comprehensive after-action review should be completed as soon as possible after the response stands down, to ensure that learnings can be captured for improvements in future responses.

The stand-down phase must commence once operational objectives have been achieved, or otherwise in accordance with advice provided by CCIMPE and agreed by the NMG. The advice that an emergency eradication operational response is no longer needed must be communicated to the affected jurisdiction(s).

Relief and recovery is a coordinated process of supporting emergency-affected individuals and communities to mitigate the impacts of a marine pest incursion. Appendix 2 in the BIMS: Marine pest version provides guidance on relief and recovery roles in the context of biosecurity incidents.

3.1.7.1 Calculating optimal sample numbers to determine when to stand-down a response

Quantification of response sampling numbers and the best time to stand-down a response are technical assessments. Advice from statisticians, ecologists, economists, or other relevant experts should be sought.

Information for calculating the optimal number of surveys to conduct after freedom is assumed to have been achieved is available from Regan et al. (2006). In many cases a decision on a surveillance program to meet the requirements of the situation may be discussed and agreed by CCIMPE. This will take into account the context of the situation and the issues around conducting a surveillance program. This simpler approach was adopted for a response to Asian green mussel (*P. viridis*) on Cape York Peninsula.

3.2 Risk assessment of potential vectors, marine infrastructure, and habitat

In the event of an emergency marine pest response, the risk status of all potential vectors, submerged infrastructure, and habitats in the receiving environment[5] should be assessed and managed if they were in the restricted or control areas during the time the marine pest was suspected to have been present.

If determined to be high-risk, vessels, marine infrastructure, surrounding habitat, and other vectors should be further assessed to determine if they require inspection and treatment. A risk assessment may determine whether this is necessary. For example, a recently cleaned vessel will have fewer marine bivalves attached than a heavily fouled vessel (MPSC 2021).

All vessels, marine infrastructure, surrounding habitat, and other vectors within the control area should be assessed and inspected for signs of the pest(s) where deemed necessary. High-risk and medium-risk vectors should be assessed and required to remain within the control area until they can be inspected and declared free of the pest as determined appropriate. Likewise, marine infrastructure and habitat in the receiving environment should be treated according to risk status.

All high-risk and medium-risk vessels that have recently left a control area should be contacted immediately if their itinerary indicates that they present a risk for spread of the pest in Australia. If the itinerary indicates visitation to another country with biosecurity requirements on vessels (e.g. New Zealand) the appropriate contact in that country should be notified. If these vessels have not entered another port or marina, they should be encouraged to remain at sea until inspection and/or quarantine arrangements can be made. Biosecurity risks detected before or during this inspection must be dealt with before the vessel can be brought further inshore. A vessel that has entered another port or coastal area should be inspected immediately. If signs of the pest are discovered, then the vessel should be directed for treatment and a back tracing of the vessel's itinerary be done and surveys undertaken of the anchorages it has visited.

[5] Marine infrastructure and habitats in the receiving environment may be naturally occurring or man-made.

3.2.1 Vessel inspection

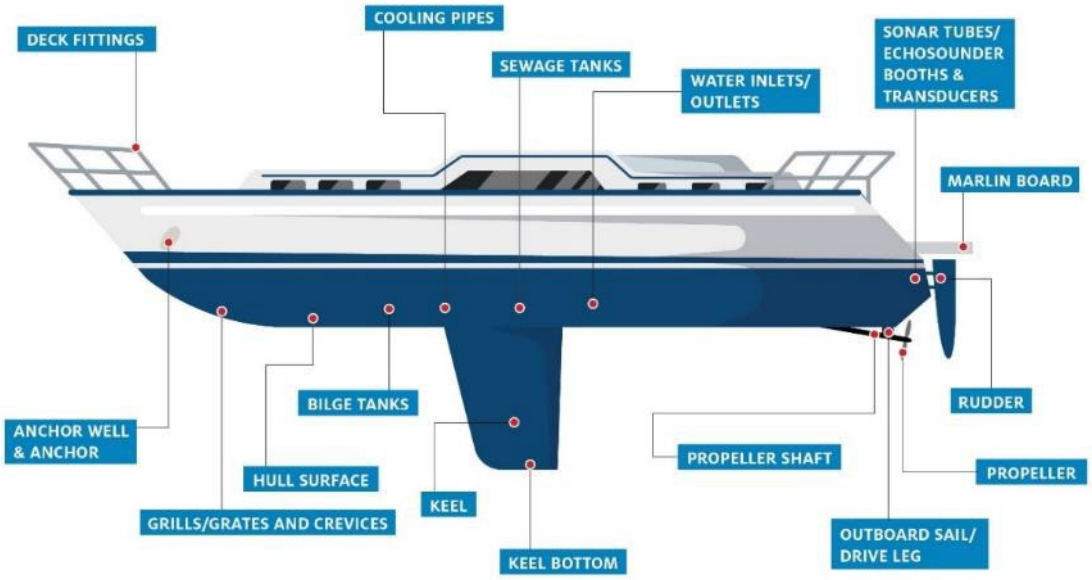
The Australian biofouling management requirements set out vessel operator obligations for the management of biofouling when operating vessels are under biosecurity control within Australian territorial seas. Bivalves can be transported in biofouling on the external hull, vessel niche areas, or within the internal seawater systems of vessels. Biofouling is likely to be greatest in wetted areas of the vessel that are protected from drag when the vessel is underway and/or where the antifouling paint is worn, damaged, or not applied.

Divers or remote operated vehicles (ROVs) should carry out in-water inspection of vessels using a standardised search protocol; see anti-fouling and in-water cleaning guidelines and International Maritime Organization (IMO) biofouling guidelines. Divers can inspect interior spaces and crevices, such as sea chests, water intakes, or outlets using endoscopes. Moist areas such as anchor wells will also require inspection for bivalves.

Critical inspection areas for vessels less than <25 metres long (Figure 5) include:

- rudder, rudder stock, and post
- propellers, shaft, bosses, and skeg
- seawater inlets and outlets
- stern frame, stern seal, and rope guard
- sacrificial anode and earthing plate
- rope storage areas and anchor chain lockers
- ropes, chains, or fenders that are in water or have been recently used
- keel and keel bottom
- sounder and speed log fairings
- live bait wells, live tanks, and deck basins.

Figure 5 Schematic diagram showing the high-risk niche areas for inspection of biofouling on small vessels <25 metres. Vessel and its components are not to scale

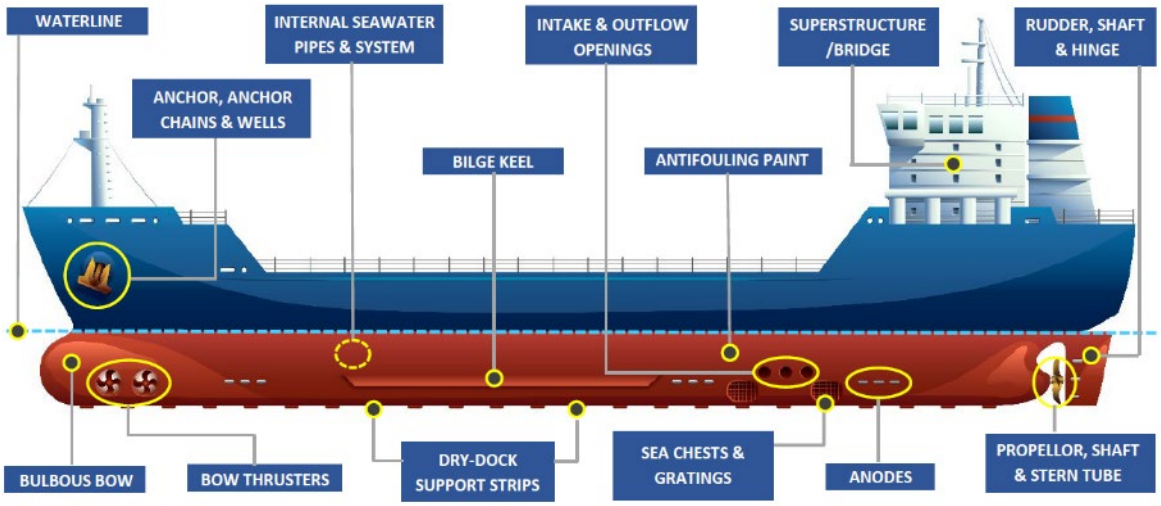


Source: Floerl (2004)

Critical inspection areas are similar for vessels longer than >25 metres (Figure 6), but include additional high-risk niche areas such as:

- sea chests and gratings
- ballast tanks
- internal seawater systems
- dry-docking support strips (DDSS)
- sonar tubes
- bow and stern thrusters
- keel and bilge keels
- anchor chain lockers
- other niches and cavities in the vessel's wet water side.

Figure 6 Schematic diagram showing the high-risk niche areas for inspection of biofouling on large vessels >25 metres. Vessel and its components are not to scale



Source: René Campbell – Department of Agriculture, Fisheries and Forestry (DAFF)

3.2.2 Inspection of marine infrastructure and habitat

Surveillance for invasive marine bivalves should be included in response measures for artificial and natural marine structures (permanent, semi-permanent, and temporary) and habitats in the receiving environment as they are at risk of being colonised by invasive bivalves. For example, the infrastructure that supports vessel operations (e.g. boat harbours, marinas, slipways, recreational boating mooring areas, and fishing ports/bases) provides hotspots for the introduction and spread of marine pests from both international and domestic vessels (MPSC 2021). The environmental conditions and artificial nature of these facilities make them highly suitable for marine pests to establish new populations once they are introduced (MPSC 2021). See Section 3.3.4 for management of marine infrastructure and habitats in the receiving environment.

3.3 Management of infested vectors and marine infrastructure

Management of infested vectors and marine infrastructure from an invasive marine bivalve will be different depending on the type of area where an infestation occurred, and the pest species in question. The following section provides specific details on the following vectors:

- vessel biofouling management
- ballast water management
- management of aquaculture stock and equipment
- management of marine infrastructure and habitat.

A summary of treatments shown to cause mortality of several high-risk invasive marine bivalves is provided in Section 5.3. These results are largely based on laboratory trials of individual or clumped organisms and will need to be adapted to ensure complete mortality on more complex structures, such as ropes or nets, or in treatment of large quantities of equipment or stock. They may also be a useful guide for selecting appropriate efficacy trials of decontamination methods for other similar

species. Table 3 below summarises management recommendations for different types of vectors which may translocate invasive marine bivalves.

Table 3 Management recommendations for different types of vectors which may translocate invasive marine bivalves

Vector	Management action
International and domestic yachts <25 m, domestic fishing vessels, ferries, tugs, and naval vessels	• Remove from water and treat and/or clean external submerged surfaces • Contained in-water treatment with appropriate biocide • Treat internal seawater systems • Treat moist places (interior spaces and crevices) • Manage ballast water (only applies to small percentage of sailing yachts) • Remove from the control area once cleaned • Educate operators and service agents of risk
Domestic commercial vessels >25 m, **and** international commercial vessels >25 m	• Inspect and treat and/or clean (if possible) external submerged surfaces • Treat or seal internal seawater systems • Treat moist places (interior spaces and crevices) • Manage ballast water • Educate operators and service agents of risk
Recreational craft (e.g. jet-skis and kayaks)	• Remove from water and clean external submerged surface • Treat and/or clean and dry internal seawater systems • Educate users and service agents of risk
Fishing gear and nets	• Remove from area and treat, clean and dry • Educate users and service agents of risk
Fouled aquaculture stock	• Remove from infested area or use an effective method for decontamination • Educate users and service agents of risk
Fouled aquaculture equipment	• Removed from infested area • Clean thoroughly by high-pressure water blast, e.g. >2,000 psi, capturing cleaned material for safe disposal • Immerse in or apply an appropriate decontamination solution (e.g. copper sulphate solution (4 mg/L) or liquid sodium hypochlorite (200 to 400 ppm) for 48 hours) • Rinse in seawater and air dry, preferably in direct sunlight • Educate users and service agents of risk
Buoys, pots, **floats**, and fenders	• Restrict movement from the control area • Treat and/or clean and dry • Educate users and service agents of risk
Water, shells, and organisms for bait or aquaria	• Restrict movement from the control area • Educate users and distributor of risk
Flotsam and jetsam	• Remove from water/shoreline **and** dry prior to onshore disposal • If possible, use barriers to prevent escape from infested area
Fauna (e.g. birds)	• Short-term permits for managing fauna can be obtained for biosecurity purposes†
Stormwater pipes and intakes	• Treat and/or clean and remove fouling • Where possible, seal until stand-down of emergency response • Educate service agents of risk

Source: Modified from Bax et al. (2002); †Fauna are recognised as vectors for spreading bivalves – for example, seabirds can forage on or collect bivalves to feed to their offspring and may inadvertently spread invasive bivalves (DAFF 2020b).

3.3.1 Vessel biofouling management

Removal of biofouling on vessels includes land-based treatment, treatment of biofouling in internal seawater systems, and various in-water treatments. Refer to the Anti-fouling and in-water cleaning guidelines for best-practice approaches for the application, maintenance, removal, and disposal of anti-fouling coatings and the management of biofouling.

For vessels known to be infested with an invasive marine bivalve, prevention of entry, treatment, or vessel cleaning before entry to a port are the most effective management options. Where suitable facilities are available and it is operationally practical, vessels and movable structures should be removed from the water for cleaning and maintenance, in preference to in-water operations. Australian dry dock facility information can be obtained from the National Maritime Centre (NMC). In-water cleaning in Commonwealth waters may require referral under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act). Dry-docking and in-water operation principles and recommendations are contained in the Anti-fouling and in-water cleaning guidelines.

If the activity does not require referral under the EPBC Act, the activity must be self-assessed using *Appendix 1: Decision support tool for in-water cleaning* of the Anti-fouling and in-water cleaning guidelines. Each state or territory jurisdiction is the primary contact for biofouling management advice. Requirements and approvals for in-water cleaning in state or Northern Territory waters differ and should be clarified with the relevant agencies as listed on the Anti-fouling and in-water cleaning guidelines webpage.

The *Biosecurity Act 2015* can be used in the absence of appropriate state or territory legislative powers and may be used in certain circumstances, including directing conveyances. The *Biosecurity Act 2015* defines conveyances as including vessels and floating structures. The Australian Director of Biosecurity (or a delegate) can authorise state and territory officers as biosecurity officers under the *Biosecurity Act 2015*, which can enable actions in a biosecurity response. A provisional list of other Commonwealth and State powers for intervention and detention of vessels is in Appendix D.

3.3.1.1 Land-based treatment

Like many fouling marine pests, invasive marine bivalves can inhabit internal piping and water intakes that are not easily inspected or cleaned. Therefore, haul-out of vessels and other non-permanent structures, such as moorings, pontoons, floats, fender moorings, chains, and ropes for inspection and treatment on land is the preferred option. This is most easily achieved for vessels <25 metres in length and where suitable haul-out and dry-dock facilities are available near the control area. Larger vessels may need to be inspected and treated in-water or suitably treated in dry-dock where possible. Hauling out vessels and large structures can of course be time consuming and expensive (Muñoz & McDonald 2014). Haul-out needs to consider locations of known marine pest infestation to minimise risks of dislodgement.

Invasive marine bivalves can withstand extreme environments and are tolerant of many treatment types. Therefore, bivalves that are dislodged during haul-out or vessel cleaning could start a new population if returned to the sea. The incident manager must approve haul-out facilities used for decontamination. Such facilities should be fully contained so that material from vessel hulls cannot accidentally or intentionally be returned to the marine environment. All macro (>1 mm) particles removed from vessels cleaned out of water should be retained and disposed of in landfill (or as biohazard material in secure landfill if appropriate). All liquid effluent (runoff) from out-of-water

vessel water blasting or cleaning must be filtered to 10 µm (Sherman et al. 2020) then collected for treatment in a liquid effluent treatment system (including municipal waste-water systems) or disposal in a secure landfill/seepage system that does not connect with waterways.

Depending on the bivalve in question, high-pressure water blasting followed by prolonged (>5 days) desiccation (aerial exposure, preferably to the sun) may also be used to treat other fouled structures removed from an infested area, such as mooring blocks, pontoons, floats, and fenders. Consideration needs to be given to likely effectiveness if using this method for invasive marine bivalves which can survive for extended periods out of the water.

Approved vessel cleaning facilities should comply with relevant jurisdictional requirements for waste containment and disposal from slipways, boat repair, and maintenance facilities. Guidance for identifying and selecting approved vessel cleaning facilities suitable for removing marine pests are given by Woods et al. (2007).

3.3.1.2 Internal seawater systems

Bivalves are robust marine organisms and capable of tolerating extreme environments. Internal seawater systems of vessels should be treated to the greatest extent possible with:

- 5% v/v industrial detergent (quaternary ammonium disinfectants) in water (preferably freshwater) for 14 hours (Lewis & Dimas 2007). In the NT an 8% QAC disinfectant is used for ten hours in internal seawater systems.
- chlorine at a concentration of 24 mg/L for 90 hours (Bax et al. 2002)
- hot water at 60°C for 1 hour (Growcott, Kluza & Georgiades 2016) or
- copper sulphate solution at a concentration of 1 mg/L for 38 hours (Bax et al. 2002).

Concentrations of chemical treatments will need checking at intervals to ensure they are maintained, particularly for chlorine which degrades rapidly in the presence of organic matter. Other treatments, especially copper sulphate, can have environmental impacts and may be regulated by legislation or by the waterway managers. Refer to Section 5.3.2.1 regarding permits to chemically treat waterways. A product using hydrochloric acid (Rydlyme®) is available and is effective at dissolving bivalves. There have been mixed reports of its effect on marine internal seawater systems (Bracken et al. 2016), but it has been used to treat invasive marine bivalve infestations in Australia.

There are novel tools that been developed to specifically treat invasive marine bivalves previously, however their broader efficacy and impacts on bivalves is unknown. A commercial biotechnology product, BioBullets, provides encapsulated active ingredients to treat filter feeding bivalves within industrial piping. Although the website does not specify internal seawater systems, they are effective at controlling bivalves in industrial piping. BioBullets offer tailored dosing programmes specific to the target organism and its environment. The active ingredients are not listed on the company website. BioBullets encapsulates active ingredients which are edible to bivalve molluscs by mimicking food items in size and buoyancy. BioBullets were effective at treating the Gulf wedge clam, *Rangia cuneata,* under laboratory conditions but their efficacy in field conditions and for treating other species is unknown (Tang & Aldridge 2019).

For further information on physical and chemical treatments, see Section 5.3.1 and Section 5.3.2, respectively.

3.3.1.3 In-water cleaning

The Antifouling and in-water cleaning guidelines state that where practical, vessels and moveable structures should be removed from the water for cleaning, in preference to in-water operations. When removal is not economically or practically viable, the guidelines accept in-water cleaning as a management option for removing biofouling, provided risks are appropriately managed and are supported by relevant jurisdictional authorities.

A variety of in-water tools use high-pressure water blasting (or jets or cavitation) to mechanically remove biofouling organisms from vessels (Inglis et al. 2013; Morrisey & Woods 2015). These tools sometimes include mechanisms to catch debris.

Depending on the location of the intended clean, there may be a range of legislative requirements for in-water cleaning in Australia waters. Applicants who wish to perform in-water cleaning in Commonwealth, state, or territory waters must first contact the relevant agency in each jurisdiction for approval. The relevant agencies are listed on the Anti-fouling and in-water cleaning guidelines webpage.

3.3.1.4 Sea chests and other vessel niche areas

Sea chests and internal seawater systems of vessels can accumulate biofouling and are structurally complex, making access for inspection and treatment difficult. Both mobile and sedentary species are found in these areas (Coutts et al. 2003). Fouling communities that include dense patches of bivalves can be attractive habitats for other marine pests. Biofouling of sea chests, internal pipework, and other niche areas can be independent to biofouling on the hull, and a clean hull does not imply clean niche areas.

Treatments of these areas for invasive marine bivalves include both chemical and non-chemical methods. There are considerations for effective in-water treatment. For instance, a key element of in-water treatments of sea chests is being able to seal off the confined spaces so that the treatment can be administered effectively. This can be achieved by sealing off external gratings using commercially available magnetic tarpaulins or bespoke sealing units. A timber ‘plug’ can be made to size to temporarily blocking off access to some vessel orifices. Sealing off confined spaces can also assist in preventing mobile marine pest species from avoiding the treatment.

For non-chemical treatments, only thermal stress can feasibly be applied to pipework and niche areas and be effective within 48 hours. The use of heated water to around 60°C will be effective against all marine bivalves, given a suitable application time, and it is also safe for the operator and the environment. *Magallana gigas* is highly resilient to thermal stress and has a reported lethal thermal tolerance of 60°C for 30 minutes or 57.5°C for 60 minutes (Piola & Hopkins 2012). A thermal treatment designed and tested on recreational internal pipework was successful at treating *M. gigas* with 100% mortality when treated with 60±2°C for 60 minutes under experimental conditions (Cahill et al. 2019b).

For most chemical treatments, such as chlorine, chlorine dioxide, bromine, hydrogen peroxide, ferrate, and peracetic acid, there is insufficient information to accurately assess their efficacy in controlling bivalves (Cahill et al. 2019b; Cahill et al. 2021). There are published reports

demonstrating that acetic acid and commercial descaler formulations such as Rydlyme®, can be effective against intact fouling assemblages within 48 hours (Cahill et al. 2019a). These preparations effectively kill attached molluscs, when appropriately contained and treated to the required levels. Refer to Section 5.3.2.1 regarding approvals to chemically treat waterways, which may require additional approvals from other jurisdiction agencies.

An important consideration for any chemical treatment is its risk to the environment and operator, weighed against its efficacy. Acetic acid and chlorine are considered safe to use within the marine environment; however, their efficacy needs to be determined. There is also concern for the effects of acidic or caustic treatments on the integrity of antifouling coatings and rust protection of vessels. Maintaining active concentrations of these chemicals requires careful monitoring. Local authorities should be contacted for requirements around use of chemicals in natural waterbodies.

Physical removal of a marine pest from niche areas is not always possible or feasible. There is risk of inadvertently releasing the biofouling organisms into the environment without significant measures to ensure that no viable material can escape. Deoxygenation and osmotic shock could take many days to several weeks to kill resilient bivalves (Cahill et al. 2019a), meaning they are unsuitable for response actions. Because thermal stress presents few unknowns for developing operational treatment protocols compared to chemical treatments, it is considered that thermal stress is the most suitable treatment of bivalves in niche areas and internal seawater systems.

3.3.2 Ballast water management

The *Biosecurity Act 2015* prohibits the discharge of unmanaged ballast water and ballast tank sediments within Australian seas (within 12 nautical miles of any land mass or in water <50 metres deep) (DAFF 2020c).

The *Biosecurity Act 2015* also regulates the discharge of ballast water and ballast tank sediments in Australian waters. Vessels intending to discharge ballast water in Australia must apply for permission via the Maritime and Aircraft Reporting System (MARS) and receive a valid Biosecurity Status Document prior to any discharge. Discharging untreated ballast water is now prohibited in Australia, unless granted an exemption by the Director of Biosecurity. The discharge of ballast tank sediment is an offence in Australia. Ballast water and ballast tank sediments are also managed by the International Convention for the Control and Management of Ships' Ballast Water and Sediments (International Ballast Water Management Convention) which has reduced the likelihood of marine pest introductions by this vector, however the risk is not removed. Australia is a signatory to the International Ballast Water Management Convention.

The approved methods for management of ballast water and ballast tank sediment can be found in the Australian Ballast Water Management Requirements (DAFF 2020c) and are as follows:

- use of an IMO approved Ballast Water Management System (BWMS)
- ballast water exchange conducted in an acceptable area
- use of low-risk ballast water (such as fresh potable water, high seas water, or fresh water from an on-board freshwater production facility)
- retention of high-risk ballast water on board the vessel

- discharge to an approved ballast water reception facility.

Note that the International Ballast Water Management Convention requires all vessels that use ballast water to comply with the regulation D-2 standard with respect to maximum amounts of viable organisms allowed to be discharged following use of an installed BWMS as of 8 September 2024. The use of ballast water exchange as a primary method of ballast water management was phased out on the same date.

3.3.2.1 Vessels arriving in Australian waters from an international location

All vessels entering Australian waters pose a potential biosecurity risk. Vessels intending to discharge internationally sourced ballast water in Australia must submit a Ballast Water Report via the Maritime and Aircraft Reporting System (MARS). The Ballast Water Report will be assessed, and a response will be issued through a Biosecurity Status Document prior to any permitted discharge. To prevent the discharge of unmanaged ballast, even vessels not intending to discharge ballast water are strongly encouraged to manage their ballast water by an approved method and to submit a Ballast Water Report. Following the first point of arrival, international vessels may uptake Australian sourced ballast water for discharge later in Australia or overseas, however there are restrictions for where Australian sourced ballast water can be discharged.

3.3.2.2 Vessels operating between Australian domestic locations

The movement of Australian sourced ballast water between Australian ports is prohibited unless it has been managed, or a low-risk exemption has been provided by the department. The approved ballast water management options are available in the Australian Ballast Water Management Requirements.

Low-risk exemptions are based on individual voyages with specific ballast water uptake and discharge locations and dates. Determination of level of risk is made via the domestic ballast water risk tables which inform the Australian Sourced Ballast Application in MARS. Any modification to locations and/or dates or additional uptake/discharge combinations by a vessel requires a new application for exemption to be submitted. Alterations to the domestic ballast water risk tables may be required in the event of an emergency response.

3.3.2.3 Vessels departing for international destinations

Vessels leaving a control area for destinations outside of Australia's territorial waters should be notified by the entity managing the control area of the risk and be required to manage ballast water as specified by the International Maritime Organization (IMO) International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 (Ballast Water Management Convention). Vessels also need to be aware of any requirements in destination countries.

3.3.3 Management of aquaculture stock and equipment

Invasive marine bivalves may be transported either on equipment used to culture marine species (such as ropes, nets, cages, buoys, and harvesting vessels) or on the stock itself. Movement of aquaculture stock or equipment from the control area during a marine pest emergency response should be permitted only if it can be demonstrated that steps taken to decontaminate the equipment and stock are able to effectively remove all life stages of the pest (i.e. 100% mortality). This should require efficacy trials of the decontamination methods and approval of the protocol by the incident manager.

Different marine pests vary in their susceptibility to physical removal or exposure to toxicants. Bivalves have strong basal attachments and/or hard exoskeletons that allow them to withstand short periods of exposure to toxicants or desiccation and are likely to be more resistant to decontamination methods than soft-bodied pests, such as ascidians or macroalgae. The effectiveness of any treatments may be affected by the conditions in which they are applied, including the ambient salinity, temperature, dissolved oxygen, pH, water flow, and the size and nutritional status of the treated species.

For all aquaculture stock and equipment treatment methods which are land-based, there is a risk that invasive marine bivalves dislodged during haul-out and may remain viable and could start a new population if returned to the sea. Containment and treatment of the waste, including influent and effluent water, may be necessary and similar precautions should be applied as per land-based treatment in Section 3.3.1.1.

3.3.3.1 Aquaculture stock

The translocation of aquaculture stock is a probable secondary vector for spread of marine pests in Australia. Species such as oysters can provide habitats that support the accidental co-transfer of other non-target invasive marine bivalve species, or potentially other pest species and parasites (Goedknegt et al. 2018). Similarly, invasive marine bivalves can settle on the shells of cultured bivalves and on aquaculture equipment.

Aquaculture stock can be treated by:

- manual removal/destruction
- detergents
- osmotic treatment.

The utility of treatment methods used to decontaminate aquaculture stock relies on the therapeutic ratio. A therapeutic ratio is the highest exposure to an effective treatment that results in no stock loss or reduced viability of stock because of the treatment (Cahill et al. 2021). To ensure survival of aquaculture stock, wide therapeutic ratios are preferred. Trials should be carried out to determine rates of mortality of the treatment on aquaculture stock and on the target marine pest (Cahill et al. 2021). Where the treatment cannot be effective, it may be precautionary to either destroy potentially contaminated stock and dispose of it to landfill, or harvest and process stock for human consumption. Thorough cleaning prior to use of another treatment will permit quicker or more effective treatment.

Import of aquaculture stock is strongly regulated and most jurisdictions have conditions on movements of aquaculture stock to manage biosecurity and other risks.

3.3.3.2 Aquaculture equipment

The protocols recommended for treatment of ropes and aquaculture equipment, such as buoys, floats, nets, and traps are:

1. Remove equipment to land, taking care not to dislodge motile species or fragments when removing structures from the water

2. Clean thoroughly by high-pressure water blasting (>2000 psi at distance of 100 mm)

3. Immerse in 2% liquid sodium hypochlorite (200 to 400 ppm) for >4 hours, or 2% detergent (e.g. DECON 90) solution for >8 hours, or hot water (>50°C) for >1 hour

4. Rinse in seawater or freshwater and air dry for >48 hours.

3.3.4 Management of marine infrastructure and habitat

All infrastructure submerged or exposed to the marine environment is at risk of being colonised by invasive marine bivalves. This includes permanent, semi-permanent, and temporary infrastructure that may be entirely or partially submerged for periods of time. For fouling organisms, marine infrastructure both artificial and natural, that cannot be removed from the water are to be considered high priority. These include, but are not limited to, structures such as:

- aquaculture infrastructure and facilities
- petroleum production and exploration industry infrastructure and facilities
- marinas, slipways, boat maintenance facilities, and recreational boating facilities
- projecting piles
- breakwaters and rock walls
- groynes
- rip-rap
- wrecks
- hulks
- hulls
- steel facings
- ropes, buoys and fenders
- moorings and mooring dolphins
- natural seabeds and reefs.

Biofouling management for infrastructure should be consistent with the National Biofouling Management Guidelines. These are available for the following industries and operators:

- aquaculture industry
- offshore infrastructure (petroleum production and exploration industry)
- port and marina operators (marinas, slipways, boat maintenance and recreational boating facilities).

4 Surveillance and delimitation

Surveillance, and specifically delimitation surveys, is used to detect invasive marine bivalve populations during an incursion and help inform management actions. Surveillance activities may occur throughout the entire life cycle of the response and will help to confirm species presence or absence, to monitor spread, to assess the size and extent of the population, to inform control programs and response objectives, or to support that eradication has been successful.

For the purpose of this manual, the following definitions are used:

- **Surveillance** – Surveillance is the systematic investigation over time, of a population or area, to collect data and information about the presence, incidence, prevalence, or geographical extent of an invasive marine bivalve, and includes active and passive approaches. Surveillance can occur before a response has been initiated or after a response has been stood down. It is sometimes called a 'detection survey'. 'Monitoring' is also a term used interchangeably with surveillance.
- **Delimitation –** Delimitation is a form of surveillance that establishes the geographic extent of an area infested by, or free from, an invasive marine bivalve during a response, and specifically informs feasibility of eradication or areas to target for control and management. Delimitation usually occurs throughout the response.

4.1 Delimitation during an incursion

After the detection of an invasive marine bivalve, a delimiting survey should be conducted quickly to establish if the area considered to be infested is localised or widespread. This information will assist in determining which response option, containment, eradication, or ongoing management, is most feasible for the incursion (van Havre & Whittle 2015). Delimitation usually occurs during the investigation phase but may also occur throughout later phases of the response to inform the next steps of the response or the status of the marine pest.

Until the response option is known, containment measures around all suspected infected area(s) should be implemented to reduce the potential spread of the invasive marine bivalve. An incursion can generally be declared delimited when no new infested area has been discovered for a period of time, given that surveys into new areas are performed to indicate spread has not occurred (van Havre & Whittle 2015).

The below section outlines considerations when planning a delimiting survey and some survey methods that may assist in delimitation, including:

- tracing an incursion (trace-back and trace-forward)
- perpendicular and margin transects
- adaptive sampling
- approach, decline, delimit (ADD).

We provide an overview of the different sampling methods for invasive marine bivalves that could be used during delimiting surveys in Section 4.3. In some cases, a sampling method is not necessarily

consistent across life stages, for instance a method that is effective for collecting larvae may be ineffective at capturing adult life stages.

4.1.1 Designing a delimiting survey

When designing and planning a delimitation survey strategy, a manager should consider:

- the allocation and management of available resources to delimit an incursion most effectively, including:
  - funding of the operation (see Section 1.3)
  - personnel and equipment (including personnel training)
  - SOPs for consistency of sample collection, preservation, and record keeping
  - ability to obtain identification confirmation from a recognised taxonomic expert or diagnostic facility.
- the location where the invasive marine bivalve was initially detected:
  - how long the bivalve has been present at the site before it was detected
  - the dispersal characteristics of the bivalve, including:
    - the frequency and quantity of reproductive output from the population since the initial incursion
    - the effects of environmental and human factors on the spread of dispersal stages.
- bivalve biology, such as survival, reproductive rate, and current stages of reproductive development
- bivalve habitat, such as distribution and suitability of potential habitats around restricted areas and control areas
- survey design sensitivity (factoring detection method sensitivity, including bivalve biology), sampling logistics, and operator safety.

Local knowledge and site inspections as well as satellite imagery, habitat suitability maps or risk maps, hydrographic charts, and online databases such as Seamap Australia can be useful for identifying areas that may contain habitats suitable for the invasive marine bivalve. Where they exist, hydrodynamic models such as Connie3 (accessed on request from CSIRO) may also be useful to simulate the likely directions of current flow. This information can provide possible rate and extent of spread of planktonic larvae from the known area of infestation (Inglis et al. 2006). Graphical summaries that plot the areal extent of new detections relative to the area searched can be used to evaluate the progress of delimitation and control of the pest (Panetta & Lawes 2005).

Knowledge of habitat requirements of an invasive marine bivalve may assist in targeting surveillance within these habitats. Habitat suitability models and particle dispersion models may also be used in conjunction to identify or prioritise survey locations (Inglis et al. 2006).

4.1.1.1 Species distribution modelling

Species Distribution Modelling, also known as Habitat Suitability Modelling or Ecological Niche Modelling, can be used to predict distributions of aquatic species (Melo-Merino et al. 2020). There are two main families of models:

- mechanistic models – where species biology is well understood (Jofré Madariaga et al. 2014)
- correlative models – require data on species presence locations, but can be applied where species biology is not well understood (Castelar et al. 2015).

For marine pest emergency responses, the Invasive Marine Species Range Mapping Tool Methodology is the preferred method. This model, developed by the Australian Bureau of Agricultural and resource Economics and Sciences (ABARES), produces a map that shows the range of the pest species in Australian coastal waters. Detail on this tool can be found in Attachment 5D outlined in the NEBRA.

Other models for predicting spread are summarised by Wonham and Lewis (2009).

4.1.1.2 Tracing an incursion

Usually conducted at the same time as delimitation, trace-back and trace-forward information is used to determine how and where an invasive marine bivalve first entered a site and where it may have spread to (van Havre & Whittle 2015).

Trace-back and trace-forward have been covered in more detail in Section 3.1.4.1 and Section 3.1.4.2, respectively.

4.1.1.3 Data sources for tracing vectors

Vessels

Tracing the movements of vessels to and from an incursion is important to know where a marine pest may have originated or be translocated within Australian waters. Some useful data sources on movements of large, registered commercial vessels are:

- Australian Government Department of Agriculture, Fisheries and Forestry
- Lloyd's List Intelligence
- MarineTraffic
- Australian Fisheries Management Authority
- Bureau of Infrastructure, Transport and Regional Economics
- Australian Border Force
- Australian Maritime Safety Authority
- local port authorities keep records of all vessel movements at their port berths and associated anchorage points.

Specific industries operating in marine environments may have information on movement of vessels and equipment such as aquaculture, natural resource extractors, maritime transport, and logistics industries. There are no consolidated data on domestic movements of smaller coastal vessels within Australian waters. Ports and some marina operators keep records of vessels that have been used in their facilities. Local industry groups, such as fishing groups, may provide point-of-contact for vessels and the movements of their respective industry sectors. Logged vessel trip reports held by the Australian Volunteer Coast Guard may also provide some data on vessel movements.

Some states and territories have developed vessel-tracking systems for a range of vessel types. For example, during the operational period of *Mytilopsis sallei* incursion in Darwin, an access database was developed that contained vessel names, contacts, current location, history of individual vessel movements and the risk status of the vessel.

Ocean current and hydrodynamic modelling

Ocean current and hydrodynamic modelling may be an effective forward and back tracing method for estimating the source and locations as part of an invasive marine bivalve response. Some tools that can assist with modelling current movements include:

- Connie3 (accessed on request from CSIRO)
- Regional Ocean Modelling System
- Marine Invader Tracking and Information System
- International Comprehensive Ocean-Atmosphere Data Set
- Global Marine Environment Datasets
- National Oceanic and Atmospheric Administration.

Hydrodynamic modelling tools often require highly specialised technical experts to operate and interpret, and thus it may not always be feasible to use such modelling techniques during a marine pest response. In addition, these tools may not have the spatial and temporal resolution required to model the hydrodynamics of specific locations such as ports, and can be quite expensive to run (see Summerson, Hester & Garaham 2018).

4.1.1.4 Perpendicular and margin transects

Allocating surveys along perpendicular and margin transects can rapidly lead surveyors to the outer reaches of an invasion, particularly at times when infestations are dense at the point of introduction and decline with distance (Hauser et al. 2016). Alternatively, survey effort could be made at the margins of the known infestation.

4.1.1.5 Adaptive sampling

Using probability-based sampling, adaptive sampling designs use sample points located on systematic grids or gradients away from the site of known infestation (Thompson 2004; Brown et al. 2013). This is most useful to ensure the greatest possible area is covered, while providing the best chance of detecting established and founding populations. The general approach is to sample at predetermined locations (often across a grid), and when the target is found, to sample more intensively near the detection (Thompson 2004). Adaptive sampling can be effective for detection of rare species, but has the disadvantages that the final sample size and survey cost are unknown prior to the survey, and field implementation may be complicated (Thompson 2004).

4.1.1.6 Approach, decline, delimit (ADD)

Approach-decline-delimit (ADD) can estimate an incursion area of a spreading marine pest (such as bivalves) in situations where the extent of spread is difficult to measure, such as when time has lapsed since initial detection or pest density is low (van Havre & Whittle 2015). The ADD approach delimits an incursion assuming very little prior information (e.g. site of first detection) by measuring the decline in density of occurrence (see Leung et al. 2010 for detail on the ADD application).

4.2 Surveillance

Biosecurity surveillance is an important part of Australia's strong biosecurity system. Surveillance is the systematic investigation over time, of a population or area, to collect data and information about the presence, incidence, prevalence, or geographical extent of a marine pest. It helps to detect and respond to biosecurity threats and provides evidence to demonstrate freedom from pests and diseases (MPSC 2019). 'Monitoring' is also a term used interchangeably with surveillance.

Australia has a coastline extending ~60,000 km and a marine jurisdiction spanning 16 million km^2 (MPSC 2019). Marine environments are susceptible to marine pest incursions which can cause significant environmental, economic, and social impacts. Early detection of new incursions enables the greatest range of management options during a response. However, due to the challenging and complex nature of the marine environment, most marine pests go unnoticed until they are established, where they can have serious impacts and become very challenging and costly to eradicate or manage (MPSC 2019). Marine pests are best managed as early as possible in the invasion process. Early detection is facilitated by robust, reliable, and practical surveillance techniques tailored to detect these pests.

The Marine Pest Sectoral Committee (MPSC) have developed the National Marine Pest Surveillance Strategy 2021-2026 (Surveillance Strategy) to coordinate Australia's surveillance activities for marine biosecurity. The Surveillance Strategy outlines nationally agreed priority requirements for enhancing surveillance of marine pests in Australia. A number of surveillance principles have been recognised and are outlined in the Surveillance Strategy, which should be followed during a surveillance program where possible. Specific activities being undertaken in the Surveillance Strategy are detailed in the National Marine Pest Surveillance Work Plan.

The MPSC is currently developing new guidelines for marine pest surveillance. These new guidelines will update information found in the Australian marine pest monitoring guidelines and the Australian marine pest monitoring manual.

This section provides a brief overview on considerations for designing a surveillance program to detect invasive marine bivalves, and key types of surveillance.

4.2.1 Developing surveillance programs

When designing and planning a surveillance program for invasive marine bivalves, a manager should consider the following key phases of its development:

- Design and planning – gathering relevant information, determining surveillance activities and methods required (including funding, trained personnel, permits, WHS requirements, and diagnostic capability), and designing a surveillance program that meets biosecurity objectives
- Implementation – standard operating procedures (SOPs) for sampling and collection techniques
- Post-sampling procedures – sample handling, preservation, quality control, analysis, and decontamination/destruction/disposal
- Reporting – includes standard datasheets and reporting instructions to maintain consistency in results
- Evaluation and review – identify improvements to be made to surveillance program.

See Table 1 in the Australian marine pest monitoring manual for further detail on the overarching guidance for developing a marine pest surveillance program.

4.2.2 Types of surveillance

The main types of surveillance commonly used during marine pest emergency responses are listed below.

4.2.2.1 Active surveillance

Active surveillance is the collection of data specifically for marine pest surveillance purposes, usually to answer certain questions (e.g. is this invasive marine bivalve present in this port?). Active surveillance is carried out in a fully structured way, such as according to formal protocols in a specified surveillance program, usually undertaken by paid staff from government or industry agencies. Many jurisdictions are now implementing their own active and targeted surveillance programs for marine pests.

4.2.2.2 Targeted surveillance

Targeted surveillance is undertaken to target specific marine pest species or taxa at certain locations and times, most of which are marine pest species of concern in a jurisdiction. Targeted surveillance is usually done as part of active surveillance programs.

4.2.2.3 General surveillance

General surveillance (also called passive surveillance) activities have one or more element(s) of opportunism, on a spectrum ranging from fortuitous *ad hoc* detections to relatively highly structured activities but excludes active surveillance. General surveillance is observer initiated. An example is a report of a suspected invasive marine bivalve by a member of the public who may be walking along the beach for recreation. General surveillance is recognised as a cost-effective surveillance tool that can be facilitated by community engagement and participation (Kruger, Ticehurst, & Van der Meer Simo 2022).

Guidelines for General Surveillance Programs relevant to all biosecurity sectors have been developed to help program coordinators, policy-makers, funders, and those who monitor and evaluate general surveillance programs to understand the key considerations for designing, planning, and implementing such programs.

4.2.3 When and where to undertake surveillance

The number of surveillance activities undertaken vary depending on the surveillance program, but usually two to four surveillance activities are undertaken each year at set locations to help capture seasonal variances. Surveillance can be done before, during, or after an emergency response to an invasive marine bivalve incursion. During an emergency response, surveillance will usually be paired with delimitation, and the frequency of surveillance may increase as a result.

Most active surveillance programs will assess high-risk locations such as ports, marinas, and naval bases, or high value locations with environmental or socio-economic value. Targeted surveillance can also be done by inspecting vessels (Section 3.2.1) and marine infrastructure and habitats (Section 3.2.2). During an emergency response, additional surveillance locations may be added, which will aid with delimitation.

Active surveillance for any invasive marine bivalve in restricted and control areas should continue until the incursion is declared eradicated or until the emergency response is stood down. If a zoning program is implemented, then it will be necessary to have targeted active surveillance for the species outside the restricted and control areas to support declaration of zones free from the invasive marine bivalve under surveillance.

If an invasive marine bivalve is successfully eradicated, ongoing targeted surveillance is still crucial as there is always a risk of re-introduction. If an invasive marine bivalve is deemed non-eradicable and the response transitions to management, monitoring and ongoing surveillance may still be undertaken for a target species because there may be interest in assessing its impacts, protecting assets, or reducing the risk of further spread.

General surveillance activities are most beneficial when observer groups have increased awareness and education on marine pests, including pest identification and reporting mechanisms. Awareness campaigns and tailored educational materials can be developed to assist observer groups with undertaking general surveillance activities.

4.3 Methods for surveillance and delimitation

This section provides an overview of the main methods used for the surveillance, including for delimitation, of invasive marine bivalves. The Australian marine pest monitoring manual and guidelines can be used to help determine quality assurance and control, and appropriate sampling intensity, for surveying invasive marine bivalves using these methods. Surveillance methods should account for seasonal variation in population recruitment or population size which may make detection by some surveillance methods more difficult.

4.3.1 Molecular diagnostics and surveillance

Molecular diagnostics and surveillance can be rapid and cost-effective tools for the surveillance and identification of invasive marine bivalves. Molecular surveillance techniques are typically highly sensitive and can assist in detecting target species, even at low abundances. Molecular methods can also be used to confirm identification of specimens when morphological identification is difficult or unresolved, or to assess population genetic structure and investigate potential source populations.

For molecular methods to effectively support marine pest management, marker/DNA probe selection, assay validation, sampling procedures, and approaches for interpretation of molecular results should be considered. A range of tools and resources exist to support molecular diagnostics and surveillance and are referenced throughout this section.

4.3.1.1 Species identification and confirmation

Invasive marine bivalves can be physically collected for molecular analysis, with the main methods of sampling being physical removal of bivalves from underwater structures and vessels by divers, or removal from intertidal structures during visual searches and on-land inspections. Bivalves growing on settlement plates may also be scraped off and later preserved (see Appendix E). For microscopic material, such as bivalve gametes, larvae, or shed DNA, plankton tows or filtered water samples can be used (see Appendix F). Preservation of bivalves is usually done with laboratory-grade ethanol to allow for molecular testing and morphological analysis. Refer to the Australian marine pest monitoring manual for details on sample collection, preservation, and processing methods for invasive marine bivalves for molecular analysis.

Molecular methods for bivalve detection and identification include species-specific assays or genetic sequencing approaches. Assays to detect specific species typically use polymerase chain reaction (PCR) with primers designed to amplify DNA of only the target species, thereby returning a detection. Fluorescent probe-based assays, either quantitative PCR (qPCR) or droplet digital PCR (ddPCR), provide the greatest specificity for detection. Some assays may detect closely related species in a genus, including native congeners, despite being designed to be species-specific. Therefore, the level of validation of an assay, including to what extent species-specificity has been established, should be considered.

Where a species-specific assay is not available, or to supplement assay results, genetic sequencing approaches can be used. Sequencing of partial genes using short-range PCR amplification and Sanger sequencing, often called 'DNA barcoding', is usually sufficient to identify invasive species with high reliability. It is recommended to amplify high-copy genes, such as the mitochondrial cytochrome c oxidase subunit I (COI) gene, or a region of the nuclear ribosomal DNA (such as 18S rRNA), however, the most important consideration is that the gene region chosen provides adequate species delineation for the taxon of interest. After quality control, DNA sequences generated can be aligned with reference sequences in databases such as NCBI GenBank and BOLD, revealing the most likely identity of the sampled bivalve. The process of routinely sequencing samples for a consistent set of genes, and adding sequences to reference databases, is commonly referred to as 'DNA barcoding'.

While many invasive taxa have been sequenced worldwide, it should be noted many species are underrepresented in databases and some genetic lineages of bivalves are taxonomically undescribed (Westfall et al. 2020). Likewise, metadata for sequences are often poorly maintained in sequence databases, which can result in misclassification – as reported for *Magallana* spp. sequences on GenBank (Sigwart, Wong & Esa 2021). Investigators should therefore be cautious when interpreting DNA sequencing results. Where possible, genetic sequencing results should be compared with morphological findings confirmed from relevant taxonomic experts to check the origin and reliability of best-matching sequences in reference databases.

Genetic sequencing or species-specific assays can be performed by diagnostic laboratories (either publicly or privately owned), universities, museums, research institutions, and some consultancies. Molecular identity can be paired with examination by mollusc taxonomic experts who can assess species identity through bivalve shell morphology. Most Australian biosecurity agencies have contact information for molecular and diagnostic laboratories, in addition to taxonomic expertise, to assist with confirming species identity during a marine pest incursion.

4.3.1.2 Molecular delimitation and surveillance

Molecular methods are used for delimitation and surveillance by testing environmental samples (e.g. water samples or plankton samples) collected from a defined area to identify the spatial boundaries of an incursion. This in turn, can assist in the prioritisation of approaches for containment, eradication, and control of bivalve populations. An invasive marine bivalve may be present at low population densities and have a heterogenous distribution, which can increase the time and resources required to undertake comprehensive delimitation, however, molecular methods are well-suited to this purpose due to their high sensitivity and low cost (Bott et al. 2010; Darling & Mahon 2011; Darling et al. 2017; Darling & Frederick 2018; Goldberg et al. 2016; Hauser et al. 2016; Trebitz et al. 2017; Zaiko et al. 2018). Molecular methods are also useful for surveillance because they can

detect life stages that are cryptic or lack diagnostic morphological characteristics, such as eggs and larvae (Darling & Frederick 2018).

In surveillance and delimitation, usually one species or taxon will be targeted, therefore species-specific approaches such as qPCR or ddPCR are recommended where available. Species-specific approaches can determine the presence or absence of the target species in a complex sample containing DNA from multiple species, with qPCR and ddPCR also able to provide data on relative abundance of the target DNA species in a sample. Sensitivity levels of qPCR and ddPCR tests are typically high, allowing detection even where target DNA is present at very low concentrations. However, where the target organism is rare, DNA may not be present in every sample. Sample quality and DNA quantity, PCR inhibition, false positive or negative errors, and seasonal variability in DNA presence can influence results (Goldberg et al. 2016). Presence of target species DNA in a sample will also depend on sample type (e.g. water, plankton, scrape, settlement plate) and sample volume.

Validated assays should be used where possible to provide confidence in molecular testing results. Assay validation quantifies assay performance (sensitivity and specificity), with laboratory validation providing data on analytical performance and operational validation providing data on field performance. The Department of Agriculture, Fisheries and Forestry developed Guidelines for development and validation of assays for marine pests that advise on consistent and comparable validation processes to develop assays. The CSIRO have also developed the Environmental DNA test validation guidelines and Environmental DNA protocol development guide for biomonitoring guidelines which provide quality control and minimum standard considerations for developing, validating, and using eDNA/eRNA assays for single- and multi-species detection. The Compendium of introduced marine pest molecular studies relevant to Australia contain species-specific information including information on validated assays. In addition, use of validated species-specific assays in combination with sampling methods of known efficacy enables calculation of the optimal sample number as part of surveillance program design. For example, the South Australian Research and Development Institute (SARDI) have developed a sample number calculator for surveys using plankton tow samples and qPCR assays.

In Australia, operationally validated PCR assays are currently available for some invasive marine bivalve species, including *Arcuatula senhousia, Mytella strigata, Perna* spp., *Mya japonica, Varicorbula gibba, Magallana gigas* and *Mytilopsis sallei*. These assays have all been validated for use in Australia (Wiltshire et al. 2023, Wiltshire et al. in press). PCR assays used in Australia should be validated for Australian conditions because of the potential for cross-reaction with native species (the majority of which have not been sequenced). Molecular information including assay validation can be found for each taxon listed in Appendix A.

Where a species-specific assay is not available, or else to characterise the species composition of a sample more broadly, high throughout sequencing (HTS), also known as metabarcoding or next-generation sequencing (NGS), may be used. HTS approaches aim to amplify all DNA sequences in a sample, with sequences then aligned with reference sequences to identify multiple species in the sample or to assess species richness and biodiversity (Darling & Frederick 2018). As for sequencing for specimen identification, the choice of barcode gene region (e.g. COI, 18S) and completeness and accuracy of sequence databases are important considerations for the interpretation of HTS data.

Managers should be aware that HTS results take considerably longer than qPCR/ddPCR due to involving a multistep process for amplification, sequencing, and bioinformatic processing.

In aquatic environments, detection probability is influenced by the decay rate of genetic material and passive dispersal from the source under local hydrodynamic conditions (Darling & Frederick 2018; Ellis et al. 2022). Positive molecular detections of target DNA do not guarantee target organisms are present and viable at the location of detection, because DNA may have been advected by water movement from source populations or present from a non-viable source such as ballast water, wastewater, or predatory excretions. Detections should be interpreted in conjunction with information on water movement patterns and potential sources of non-viable DNA. Historic environmental samples can also be tested to improve temporal surveillance resolution and assist in trace-back activities.

Positive detections using molecular methods should be confirmed using traditional surveillance methods where possible (e.g. settlement arrays or visual surveys), however, traditional surveillance methods may lack the necessary sensitivity to confirm occurrences of species at low abundances. Positive detections may be the result of false positives which can occur due to a lack of assay specificity or to sample contamination. The use of validated assays minimises the risk of specificity issues, while careful sample handling and good laboratory practices should be applied to minimise contamination risks. The use of appropriate negative controls can assist in determining whether contamination has occurred. Where suitable data on sampling method and assay performance are available, occupancy modelling approaches can be used to aid interpretation of molecular surveillance results (Burian et al. 2021; Wiltshire 2023).

4.3.1.3 Molecular ecology, population genetics/genomics, and source attribution

By sequencing many individuals among sample locations, researchers can use population genetic/genomic and phylogenetic methods to investigate the genetic diversity of invasive marine bivalves. In turn, patterns in genetic diversity can be used to estimate relationships and the origin of incursions, leading to source population attribution. Population genetic variation can also be analysed to estimate demographic variation, including variables such as effective population sizes and inbreeding which can indicate the long-term viability of sexually reproducing populations. Genomics and transcriptomics have also aided in understanding evolutionary adaptations of invasive marine species in different locations. These approaches are more costly than simple genetic identification methods, and require considerable time and bioinformatic expertise for analysis, but they can provide valuable insights to determine the potential origin of outbreaks, modelling invasion pathways, and assist with the management of established invasive populations (Darling et al. 2017; Sherman et al. 2016; Viard et al. 2016).

While still expensive, costs for long- and short-read DNA sequencing have decreased considerably in the last decade, and it is therefore feasible to consider using genomic sequence data for invasive marine bivalves. A reference genome (and genomic or transcriptomic data for multiple individuals) can provide valuable information to improve the surveillance and the long-term management of high-risk taxa. For example, genomic data can be used to develop new, more effective primers for species identification and detection, and reference genomes can significantly improve the resolution of metagenomic and population genomic methods. Whole-genome sequencing itself also provides the highest level of resolution for source population attribution (Viard et al. 2016).

4.3.2 Divers and remote operated vehicles (ROVs)

Divers and ROVs are forms of underwater visual surveys. In shallow, enclosed waters, underwater surveys may be performed by snorkellers. Divers and ROVs may be used for both surveillance activities and delivering treatments for invasive marine bivalves during instances when these methods are deemed most appropriate.

To collect evidence of invasive marine bivalves, divers and ROVs can take photographs or videos of suspect specimens. This technique is cost-effective, but is highly limited by water visibility, which can prevent accurate species identification. Divers can collect bivalve specimens by hand, which can then be used for later taxonomic or molecular analysis. Some models of ROV also contain motorised arms which can take physical samples. Taking physical samples is limited by accessibility and safety, but provides quality assurance if visual techniques are inadequate.

Divers can be particularly effective at detecting invasive marine bivalves that tend to aggregate around complex structures such as jetty pylons. However, the ability to observe an invasive marine bivalve while diving relies heavily on water visibility, identification training, safety, and search techniques. Divers can use touch very effectively to detect some bivalves in inaccessible niches. On several occasions, mussels have been detected in vessel niche areas by divers using touch.

Cost of professional divers needs to be considered by managers. If visibility is low, or if there are safety and access issues to the site, then visual surveys will be compromised. These same visibility limitations apply to ROVs. However, ROVs can be used in place of divers, particularly in confined spaces (e.g. areas near marinas) or when hazards are present (e.g. crocodiles, sharks, stinging cnidarians). The use ROVs in marine pest surveillance is still being optimised and few data are available on their effectiveness. ROVs can have a significant learning curve to use, especially with several makes and models on the market. In addition, they can be very heavy and challenging to deploy, and prices may exceed >$35,000 AUD (Ellard 2021).

Divers are required for the application of several treatments, as well as for subtidal surveys, around wharf piles, vessels, floating pontoons, and other artificial structures in port and marine environments, and on intertidal and shallow subtidal reefs. Treatments deployed by divers include:

- underwater vacuum, suction, and filtering systems
- in-water cleaning and grooming machines
- wrapping and encapsulation
- smothering
- osmotic treatment.

Divers were effectively used to collect and search for *P. canaliculus* after its introduction into the Gulf of St Vincent, South Australia, in 1996. A mature population of 12 to 24 mussels were collected during a research dive, with subsequent diving and dredging finding only one more mussel in areas expected to be colonised by a reproductive event from an established population (McEnnulty et al. 2001). It should be noted that the population of *P. canaliculus* in South Australia could have died out naturally as well as through human intervention, but this example highlights that early detection and removal of small populations can occur with minimal resource expenditure.

Divers are regularly engaged in treatment of marine pests on visiting vessels. Infestations of *M. sallei, P. viridis* and *M. strigata* on vessels have been effectively managed using divers who may selectively treat particular areas of the vessel or remove isolated patches of infestation.

4.3.3 Settlement arrays and plates

Settlement arrays, typically comprising arrangements of settlement plates held in different orientations, are commonly used to study recruitment of sessile marine organisms from planktonic life stages, such as larvae, into a benthic or sessile juvenile or adult phase. Settlement arrays are unlikely to be an effective sampling method for infaunal bivalves such as *Potamocorbula amurensis* and *Mya arenaria,* but are much more likely to be an effective sampling method and surveillance tool for epifaunal fouling species like mussels and oysters. Given that many epifaunal bivalves spread via biofouling, settlement arrays are a practical, low-cost method to detect fouling species.

Settlement arrays have many advantages and are commonly used for marine pest surveillance as a result. Advantages of settlement arrays include:

- being cost-effective to make
- simple to use and easy to deploy by non-specialists
- can sample species continuously over a long period of time (temporal scales)
- can be deployed in different areas and depths of the water column (spatial scales)
- can sample species inaccessible to divers or other sampling methods because of organism size
- fouling organisms growing on plates can be scraped and used for both taxonomic identification and molecular diagnostics
- give an indication of native fouling species seasonal abundance.

Settlement arrays may provide early detection of incursions when applied in routine monitoring, but are typically unsuitable for delimitation or to assess pest status following attempted eradication because arrays need to be deployed for a period of weeks to months in order to collect recruiting bivalves. Another disadvantage of settlement arrays is the relationship between the presence and abundance of the target species within the environment and its detection on the settlement surface is complex and difficult to quantify, which is similar to other methods of passive sampling. For invasive marine bivalves, this means that:

- uncommon (rare) biofouling species, including those that are at an early stage of a population's establishment, will be under-sampled
- other more abundant species may establish on the arrays and prevent biofouling species from settling due to competition for space
- absence from an array does not necessarily mean the absence of an established population because of species-specific variation in settlement preferences. For example, settlement arrays deployed following the introduction of *Perna viridis* in South Carolina, USA, failed to detect any *P. viridis* despite numerous individuals found through visual searches (Knott et al. 2008).

Variables which influence the recruitment of organisms onto settlement arrays include: timing of deployment, duration and depth of deployment, orientation and shading of the surfaces, surface

rugosity and material, water currents and tidal movements, predation, and the presence of antifouling coatings (Tait et al. 2016). The number of settlement arrays or surface area of settlement substrata must be relatively high, and the settlement area must be attractive for settlement of the target bivalve (Floerl et al. 2012). The species' biology and life habit will also influence settlement onto arrays, e.g. epifaunal bivalves will likely settle onto arrays, but infaunal bivalves will not.

Various designs of settlement arrays have been used for marine pest surveillance (Floerl et al. 2012; Sutton & Hewitt 2004). Refer to Appendix E for a summary of potential settlement array designs for sampling invasive marine bivalves. The box settlement array design has been extensively tested and is the result of ongoing work by WA. This type of array is now used across Northern Australia where tidal energies permit.

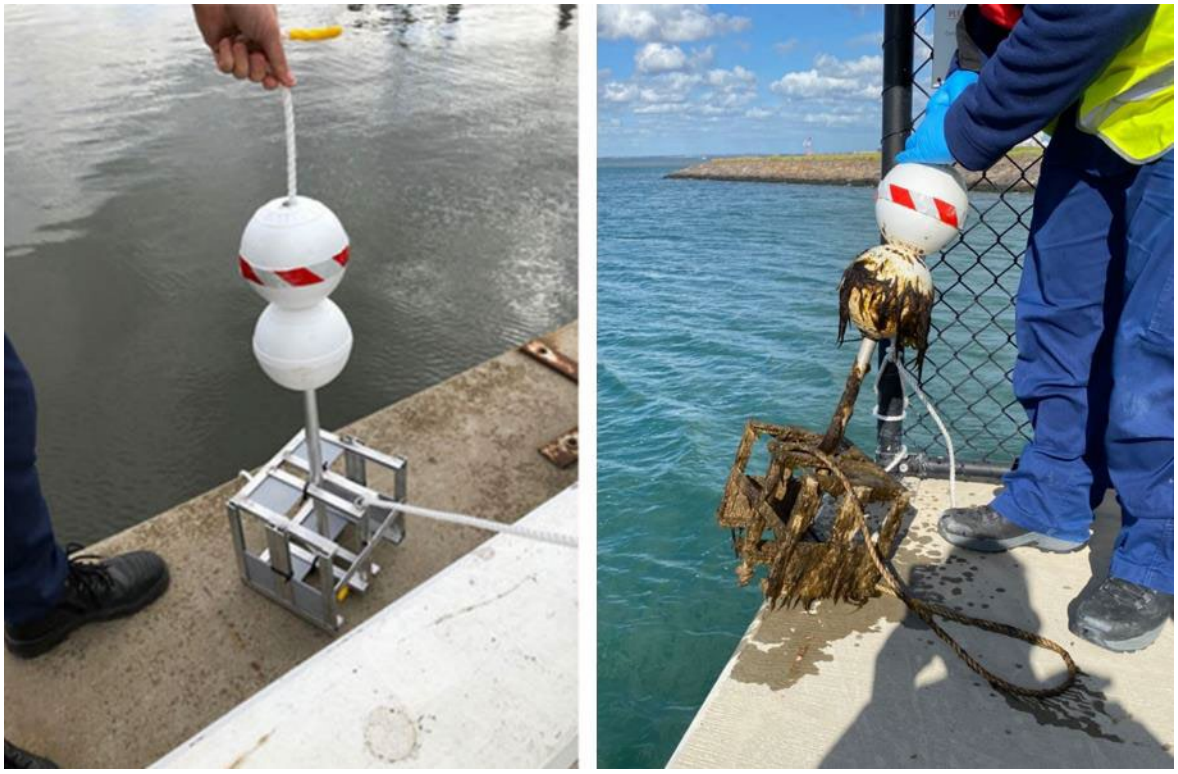
Generally, settlement arrays consist of a collection of plates of varying materials and surface features that act as settlement substrata for larval phases of sessile marine species (Photo 1). Arrays are usually placed about 2 metres below low tide and attached to a fixed structure in the environment such as a wharf piling. Where tidal amplitudes are large, a suspended array to maintain a constant depth is essential. Settlement arrays are typically deployed for a minimum of three months to allow biofouling to reach a size and maturity to enable effective taxonomic identification. Material scraped from settlement plates can alternatively be tested using DNA-based methods such as PCR or HTS.

Different orientations of settlement plates and variations in depth and numbers of settlement arrays deployed can be used (Tait et al. 2016). Settlement arrays can also be deployed in a staggered manner to enable continuous sampling over the reproductive period of the target bivalve while minimising overgrowth of biofouling organisms. For example, two months after deploying a settlement array, a second settlement array could be deployed. After four months of deployment the original settlement surfaces are retrieved while the second set of surfaces is retrieved two months later. This allows for two overlapping deployments each of four months' duration.

Plastics (PVC, Perspex), wood, cement/rock, metal (steel aluminium) and fibreglass have successfully been fouled by invasive marine bivalves (Tait et al. 2016; Vekhova 2006). The likelihood of a bivalve to settle will differ between settlement surfaces and this needs to be considered. For example, natural rope or other filamentous fibres are used to promote *P. canaliculus* settlement in aquaculture settings in New Zealand.

Settlement arrays are the recommended surveillance method for locations with high-risk vectors, such as international or domestic vessels (McDonald et al. 2019). In Western Australia, the implementation of the State-Wide Array Surveillance Program (SWASP) which uses settlement arrays combines collaboration with the Western Australian Government, port and marina authorities, and industry partners, which has been beneficial to the surveillance program (Kruger, Ticehurst & Van der Meer Simo 2022).

Photo 1 Box settlement array design showing square plates attached to a frame (left), and settlement array covered in biofouling after immersion in water (right)



Source: Agriculture Victoria

4.3.4 Plankton tows and water samples

Plankton or water samples can be collected using plankton nets or water containers to test for eDNA of target bivalve species. In some cases, plankton or water samples can also be examined under the microscope to morphologically identify larval stages. Many invasive marine bivalves will have planktonic larval stages or shed DNA into the water column (e.g. gametes after spawning), and so water samples can be used as a surveillance method for planktonic life stages.

Plankton tows, sometimes called plankton trawls, are commonly used to sample planktonic organisms or their DNA from the water column, including bivalve larvae and gametes. The samples are collected with a plankton net that is pulled through the water column either vertically, horizontally, or obliquely. The net is usually deployed from a vessel, but tows can also be performed from wharves or pontoons. See Appendix F for an example method of using plankton tows for detecting invasive marine bivalves. Further detail on plankton and water samples, as well as other methods for marine pest surveillance, are located in the Australian marine pest monitoring manual.

Plankton tows have the benefit of being able to concentrate material from large volumes of water, which can improve detections of DNA at low concentrations and overcoming patchiness (Bowers et al. 2021). However, plankton nets may be susceptible to cross-contamination, poor sterilisation, and challenging field logistics in some environments. Giblot-Ducray and Bott (2013) developed a plankton sampling protocol for molecular testing of marine pests, including for bivalves. This method was further assessed and refined by Deveney et al. (2017) and has been subsequently applied to

molecular surveillance around Australia (Wiltshire et al. 2019a,c; Wiltshire et al. 2020; Wiltshire et al. 2022).

Containers can be used to collect water samples, such as Niskin bottles and van Dorn "horizontal" samplers, which can collect water from discrete depths (Bowers et al. 2021; Ellis et al. 2022). Due to the volume and turbidity of water collected with these containers, water samples will usually need to be filtered. Consideration needs to be given to the filtration methods used, among other variables, which are outlined in the Environmental DNA protocol development guide for biomonitoring guidelines. Another method for sampling eDNA from water samples is the Smith-Root eDNA water sampler which is used in Victoria, Queensland and New South Wales.

Samples collected with plankton nets and containers can be filtered and tested for eDNA, which is useful for microscopic organisms which may be difficult to identify morphologically, or for gametes (i.e. eggs and sperm). Sensitivity levels of PCR tests are high, allowing detection even where target DNA is present at very low concentrations in the water sample. However, where the target organism is rare, DNA may not be present in every sample. In addition, samples may only collect planktonic life-stages or shed DNA of a target species at certain times (e.g. after spawning), and therefore timing of surveillance should be considered. When surveillance is outside likely spawning periods, plankton tows may be supplemented with other methods to improve the likelihood of detection, with the specific method dependent on the bivalve species.

4.3.5 Visual shore searches

Visual shore searches are usually undertaken in the intertidal zone or on maritime infrastructure, such as pontoons, jetties, or wharves. Visual shore searches may be used as both a surveillance activity and to deploy certain treatment methods (e.g. physical removal of invasive marine bivalves by-hand). Visual shore searches are commonly undertaken during both active and general surveillance activities.

Visual shore searches are sometimes confined to a set time or area limit. A standard visual shore search may involve 10-minute timed searches along a transect or be based on the number of rocks/boulders searched. Other forms of visual shore searches may be unconstrained by time or area. Once searchers are familiar with the identity of the target bivalve species, then many searchers can be deployed, covering large areas. Some invasive marine bivalves inhabit the intertidal zone or foul on easily observed structures such as buoys, pontoons, and ropes, which allows for easy visual observation by searchers (Photo 2). Like divers, searchers can take photographs or physically collect invasive marine bivalves for identification.

Photo 2 Visual shore search showing *Magallana bilineata* shells on beach rocks in Cooktown, QLD

Source: Evan Rees, Northern Australia Quarantine Strategy (NAQS)

Visual shore searches are limited by weather conditions and site accessibility. Complex or inaccessible habitats such as mangroves and steep cliffs, areas with high boat traffic or swell, or dangerous marine animals (e.g. crocodiles) can impede visual shore searches. Access to maritime infrastructure like certain marinas or commercial wharves can be dependent on having the appropriate permissions, permits, and PPE to undertake the search. Coordination to take advantage of the lowest tides in the year may assist in targeting a wider range of intertidal habitats. Often visual shore searches are used to augment other sampling regimes, like settlement arrays and eDNA water sampling.

5 Containment, control, and eradication

Containment, control, and eradication may be attempted after the confirmed detection of an invasive marine bivalve. Containment is a component of control which aims to restrict an incursion of a marine pest to a limited geographical range. Eradication aims to eliminate a marine pest from the infested area. The feasibility of containment or eradication of an invasive marine bivalve will depend on the nature and location of the incursion, the response objective, and the management option(s) adopted.

Management options usually include:

- containment and control of the invasive marine bivalve to the infested areas and prevention of further spread; incurs ongoing costs and efforts, or,
- eradication of the invasive marine bivalve from an infested area; incurs highest initial control measure and cost.

For the purpose of this manual, the terms 'containment', 'control' and 'eradication' have been adapted from the National Biosecurity Committee and are outlined below:

- **Containment** – The application of measures in and around an infested area to restrict the spread of an invasive pest to a defined region. This may include reduction of the density or area of the infestation where appropriate or managing vectors. A containment program may include eradication of satellite infestations.
- **Control** – In relation to organisms, control actions are those which aim to reduce the number of pest organisms, prevent an increase in pest numbers and spread, reduce organism activity to limit their impact, or modify the behaviour or characteristics of the pest population. Control may involve partial eradication or other actions which limit population size and/or reproductive potential. This term is sometimes used interchangeably with 'management.'
- **Eradication** – Under the NEBRA, eradication in relation to pests means eliminating the pest from an area. Eradication is indicated by the pest no longer being detectable.

For methods suitable for containment, control, and eradication of invasive marine bivalves, see Section 5.3.

5.1 Containment and control

Containment aims to prevent secondary spread and assists to maintain the possibility of eradication of an invasive marine bivalve. During an emergency response, containment should be attempted as soon as possible after the incursion has been detected. If a decision is made to implement containment and control, then the incident manager will (in consultation with stakeholders) recommend that interim containment measures be implemented to minimise the risk of bivalve translocation from the infested waterway. This may include movement controls of potential vectors, public information campaigns, policies and practices for vessel equipment sanitation and surveillance, and control of secondary infestations outside the infested waterway (see Section 3.3).

Containment may also include control methods to help reduce population density or area infested by the invasive marine bivalve (see Section 5.3. for control methods).

National Control Plans (NCP) have been developed for several marine pests that are already established in Australia which may have the potential to spread further or cause negative impacts. The purpose of the NCPs is to deliver an agreed national response to reduce impacts and minimise spread of agreed pests of concern. An NCP exists for invasive marine bivalves *Varicorbula gibba* and *Arcuatula senhousia.* These NCPs cover:

- practical management actions and cost-effective approaches to control or reduce the impact of the marine pest
- recommendations for future research and development, including a benefit-cost analysis and planning tools
- recommendations for additional public information and education strategies
- an implementation strategy.

5.2 Eradication

Eradication of any invasive marine bivalve requires complete elimination of the species from the infested area. Eradication programs will be more successful if initiated early and if the programs are well designed and resourced. In addition, eradication is more likely to be successful or feasible if initial investigations determine that the species is not widespread, can be contained, is not difficult to detect, or is present or potentially present in closed/semi-closed environments. An eradication program is more likely to be successful if it has broad public support and reduced risk of being compromised (e.g. negative media releases). There are no examples of a program that has successfully eradicated an invasive marine bivalve from a widespread population, however localised eradications of small populations have been achieved.

Eradication is the preferred response option when:

- the bivalve can be determined to be technically feasible to eradicate
- discounted benefit-cost analysis favours eradication over management
- the socio-political environment supports using eradication methods.

The National Environmental Biosecurity Response Agreement 2.0 (NEBRA) establishes national arrangements for responses to nationally significant biosecurity incidents when there are predominately environmental or public benefit. The NEBRA provides a mechanism to share responsibilities and costs for a response when eradication is considered feasible, the pest is considered to be of national significance, and the response is calculated to be cost-effective.

A known example of a successful eradication of an invasive marine bivalve is from Australia where the black-striped false mussel, *Mytilopsis sallei,* was eradicated from three marinas in Darwin in 1999 (Willan et al. 2000). Other small, localised incursions of marine bivalves have also been successfully eradicated. For instance, the population suppression and eradication of *P. perna* from New Zealand (Hopkins et al. 2011) and the early detection and removal of a small population of *P. canaliculus* in South Australia. Detection and removal of *P. perna* and *P. canaliculus* are examples

of how early detection can result in eradication at a minimal resource expenditure, whereas the eradication of *M. sallei* was assisted by being contained to a semi-closed environment. While it is possible these bivalve populations could have died out naturally, perhaps due to changes in salinity as argued by Wells (2019), it is crucial that managers act on bivalve incursions as early as possible during a biosecurity response, as there is no guarantee an incursion will die out naturally. The biology and reproductive strategy of an invasive marine bivalve will influence the effectiveness of an eradication program. Due to spread by tides and currents, eradication may not be possible in open coastal waters where there is high movement of water.

Eradication is most likely to be feasible when:

- the infestation is detected early and controlled before spawning can occur
- the area inhabited is small, that is, <1,000 m^2
- the infestation occurs within an area of minimal flushing or exchange of water
- the available habitat occurs in relative shallow water, such as <15 m
- the population is relatively aggregated and has not yet reached reproductive maturity (Crombie et al. 2007).

Tracking the success of eradication to ensure effectiveness of response management can inform the next steps of the response. Expert modelling can give a measure of progress during an eradication program. For example, an 'eradograph' uses the specific characteristics of the marine pest and the incident managers' eradication objective to generate the temporal trajectories of delimitation. It can imply the reallocation between search and control activities or to discontinue, maintain, or increase an eradication program. However, any applications or suggestion of changes in an eradication program must be evaluated using a benefit-cost analysis (Burgman et al. 2013).

In planning an emergency eradication response, it is important to obtain good descriptions of the nature of the incursion, including the environment in which the pest is located and the distribution and abundance of the pest. As much as possible, these descriptions should be spatially explicit (that is, geo-referenced) to guide application of treatment methods.

Seasonal conditions and sexual maturity of the initial invading population can determine if there is sufficient time to eradicate a population before mature individuals are able to spawn. Because spawning may be trigged by relatively sudden changes in temperature, salinity, or stress, or by seasonal factors, there may be a short period when eradication may have a reasonable chance of success before spread occurs. This is particularly true for invasive species on visiting vessels which may not be in spawning condition when arriving or may be immature, meaning that visit duration is important in determining a course of action.

Table 4 summarises the variables that may be used to describe the nature of an invasive marine bivalve incursion and help define likelihood of eradication.

Table 4 Variables to describe the nature of the invasive marine bivalve incursion

Variable	Distribution level
Area currently infested	Very small (<100 m^2) Small (100–1 000 m^2)

Variable	Distribution level
	Medium (1 000–10 000 m²)
	Large (1–10 ha)
	Very large (>10 ha)
Abundance	Low
	Moderate
	High
Pattern	Continuous
	Fewer than 5 patches
	5 or more patches
Use of suitable habitat	Low (<10%)
	Moderate (10–50%)
	High (>50%)
Maturity of organisms found	Juveniles
	Sub-adults
	Adults
	Adults (sexually mature)
Maximum depth of infestation	Shallow (<2 m)
	Moderate (2–15 m)
	Deep (>15 m)
Maximum depth of available habitat	Shallow (<2 m)
	Moderate (2–15 m)
	Deep (>15 m)
Turbidity	Clear (visibility >5 m)
	Moderate (visibility 1–5 m)
	High (visibility <1 m)
Water exchange in incursion area	Minimal
	Low
	High

Source: Table modified from Crombie et al. (2007)

5.2.1 Infestations in open coastal environments

There are limited emergency eradication response options available for invasive marine bivalves in open coastal environments, particularly on high energy coastlines or water >10 metres deep. Many treatment and control options described in Section 5.3 may be applied to small-scale incursions in the open ocean environment. The primary difficulties, however, are containing the wide dispersal of larvae if reproduction is occurring and maintaining treatment conditions at a lethal level for enough time to be effective. For instance, the application of chemicals will require development of support structures or technologies to account for current and wave action affects. Most chemical treatments also cause impacts on non-target species and may have significant environmental effects that require consideration.

Successful eradication of small incursions may be possible using methods such as physical removal, smothering, small-scale containment, and chemical treatment if the incursion is detected early or where site- and species-specific conditions allow removal or containment of the bivalve species.

Successful eradication usually combines a range of methods, some of which may be selected on factors such as population distribution and density (Green & Grosholz 2020).

Many invasive marine bivalves have high fecundity and long planktonic larval durations of several days up to multiple weeks, allowing larval spread over large distances by tides and currents. Because of this, eradication may not be possible in open coastal waters where there is high movement of water. Priority invasive marine bivalves to Australia take varying times to reach sexual maturity. *Mytilopsis sallei* and *Perna* spp. take between 1 and 3 months to reach sexual maturity whereas *Mya arenaria* can take up to five years (Abraham & Dillon 1986).

5.2.2 Infestations in closed or semi-enclosed coastal environments, and on aquaculture stock and equipment

Eradication is most achievable in closed or semi-enclosed coastal environments, such as some marinas and coastal lakes, or from aquaculture stock and equipment because the bivalve population can be more easily contained, and it is possible to maintain conditions necessary to achieve mortality. Various treatment options are possible in these circumstances, including application of chemical biocides, physical removal, and smothering through wrapping and encapsulation. Chemical biocides and physical methods have both been used to successfully treat a bivalve population (Ferguson 2000; Hopkins et al. 2011). In both cases, timeliness was essential, because if bivalves have spawned and the larvae have settled, then control will be far more difficult. Repeating treatment methods over the species' known or suspected recruitment period may be required.

If the infestation is confined to a relatively small, enclosed, or semi-enclosed waterway, it may be possible to treat the entire water body and all aquatic habitats within it (Willan et al. 2000). However, consideration must be given to native species and habitats in the water body which may be affected by these treatments. Similarly, if the infestation is confined to specific aquaculture equipment or stock then it is possible to treat the equipment or dispose of the infested stock. If this is not possible, then the management success will depend more heavily on delimitation surveys and active surveillance to locate and treat all clusters of the bivalve population.

The wide range of physical tolerances of invasive marine bivalves presents many challenges for their control. Bivalves are particularly tolerant to several treatment options because of their ability to withstand extreme environments and withstand desiccation. For example, *M. gigas* is particularly tolerant to treatment methods, often requiring prolonged exposure or high concentration dosage to achieve the desired outcome. Other bivalves, such as *M. sallei*, may be more susceptible to treatments, in part because they have a thinner shell.

In areas where bivalves have successfully invaded and become established, complete eradication may not be achievable. It may be more feasible to focus on control where the goals are to reduce population densities down to levels where they reduce the impact on ecological functioning of the system ('suppression') and to minimise the spread to other areas ('containment'). Control will require continued coordination and communication between affected parties. When resources allow, all habitat potentially suitable for the pest should be surveyed and treated where required. When this is not possible, habitats should be prioritised based on suitability for the bivalve species and delimitation survey results.

5.3 Methods for containment, control, and eradication

Methods and treatments that have been used for containment, control, and eradication of invasive marine bivalves are listed in this section. The methods used to treat invasive marine bivalves will vary in efficacy according to the size and location of the incursion, the pest's biology, the capacity and resources of response personnel, and whether the population is in open, closed, or semi-enclosed coastal environments. These methods can be used at any phase of a response for which they are determined most appropriate for containment, control, or eradication. More detail on the efficacy of these treatments can be found in summaries by Aquenal (2007) and McEnnulty et al. (2001).

The methods used to contain, control, or eradicate invasive marine bivalves can be divided into three generic treatment types:

- physical treatment
- chemical treatment
- biological and ecological control.

Methods for sampling and controlling invasive marine bivalves include physical removal (including harvesting), chemicals, biocontrol, and environmental remediation. The acceptability of control methods depends on their feasibility, effectiveness, cost, public support, and off-target effects. For example, physical removal may only be appropriate for incursions that occupy relatively small areas and are inappropriate for large scale control.

The biology and ecology of invasive marine bivalves also needs to be considered when selecting appropriate control methods. The efficacy of the control method can be impacted by the ecology of the bivalve (i.e. controls for infaunal bivalve species will likely be different to controls for epifaunal bivalve species). Public information and engagement to key stakeholder groups must also be considered as a high priority; if there is a lack in public support for a certain method, this may compromise the biosecurity response.

The three broad categories on methods to treat invasive marine bivalve populations are summarised in this section. Table 5 presents examples of control methods used for high-priority invasive marine bivalve species to Australia. Generally, younger life stages are likely to be more susceptible to most treatments presented below and this should be taken into consideration when assigning treatment.

5.3.1 Physical treatment

Physical treatments include a range of methods that rely on the ability to detect and either remove marine bivalves or kill them *in situ*. Physical treatments are generally the most socially and environmentally acceptable way of removing marine bivalves from a system. Physical treatments can be difficult to achieve in complex habitats often inhabited by marine bivalves, such as oyster reefs or mangrove forests, or in high-traffic areas such as wharves, making operations challenging or environmentally destructive. Consequently, physical treatments are mostly effective in small and accessible areas, such as on a relatively flat seabeds or on artificial structures, such as a hull surfaces in a contained marina.

5.3.1.1 Manual removal

Manual removal typically refers to collection and removal of the pest organism by hand or by using handheld implements. Manual removal has been used as a rapid response and long-term control option for some introduced macroalgae, molluscs, seastars, and crabs (McEnnulty et al. 2001). Hand collection of invasive marine bivalves can be achieved underwater by divers, or via removal from artificial structures on land (e.g. vessels and aquaculture equipment) and the intertidal zone (rocky shoes). Manual removal can be particularly effective where an infestation on a vector (e.g. a vessel) needs to be eradicated before spawning and spread can occur. Manual removal has been effectively used to minimise risks when large mussels have arrived on vessels.

The advantages of manual removal are selectivity for the target bivalve and limited damage to non-target species. Manual removal is also useful for cleaning niches and areas that are challenging to reach with other cleaning equipment (Morrisey & Woods 2015). However, manual removal requires visual detection of the pest and cannot be applied effectively in turbid underwater environments where such detection is impaired. Manual removal is of greatest utility when incursions are small and spatially confined or when they are in sensitive environments (such as marine reserves or areas of high biodiversity value) (Morrisey & Woods 2015).

Manual removal by divers is most effective as a control method in small areas underwater, such as in areas around new incursions, and shallow water depths <12 metres. *Perna canaliculus* was eradicated from a shipping channel to the outer harbour wharf in the Gulf of St Vincent, South Australia in 1996. A mature population of 12 to 24 mussels were collected during a research dive, with a subsequent dive and dredge found only one more mussel in areas expected to be colonised by a reproductive event from an established population (McEnnulty et al. 2001). The success of this eradication operation was influenced by the localised nature of the incursion, and it should be noted that this population may have died out from natural causes.

Oyster farmers in New South Wales have used hammers to smash *M. gigas*, to control feral populations and the oyster virus ostreid herpesvirus-1 microvariant (OsHV-1 µvar) (Cavanagh 2014). However, manual destruction of >100,000 wild *M. gigas* using hammers in South Australia showed no evidence of successfully controlling the population or limiting its spread (Keen 2010; Sierp 2019). This rudimentary method of destruction could have also unintentionally released gametes from the crushing. This method of control could be more effective on a small scale for juveniles and at a time of year when bivalves do not have viable gametes.

5.3.1.2 Mechanical removal

Mechanical removal entails use of machinery to directly remove the target species and may involve techniques such as mowing, dredging, trawling, or mopping. Mechanical removal will increase the area capable of being treated compared to manual removal. Dredging was used to effectively eradicate *P. perna* from New Zealand following accidental introduction after de-fouling of a drilling rig. Over 200 tows using a commercial scallop dredge (width 2.4 metres and 40 mm mesh size) covering approximately 94% of a 12.6 hectare subtidal area collected 35 tonnes of material defouled from an oil rig to reduce the density of *P. perna* to below 10 individuals per m^2, which is below the level where successful reproduction is likely (Hopkins et al. 2011). Eradication of *P. perna* from New Zealand was also assisted as the marine bivalve was in a sub-optimal habitat (Hopkins et al. 2011). Dredging to collect other infaunal bivalves such as *A. senhousia* may be possible on a small scale.

Byssal threads of *A. senhousia* intertwine, creating large mats within intertidal mudflats of estuaries and sheltered bays. However, *A. senhousia* may also be found as a fouling organism on pylons and panels where they do not use byssal nests (Willan 1987), so physical removal by dredging may only be one component of a successful eradication effort.

The efficacy and environmental impact of mechanical tools should be considered when implemented as part of a management response. Some of these practices can cause considerable bycatch or ecological damage, either through direct disturbance of the assemblages or through modification of habitat (e.g. removal of habitat-forming species, increased turbidity, release of toxic chemicals from the seabed).

5.3.1.3 Harvesting

Harvesting can reduce numbers of some invasive marine bivalves with community assistance programs, recreational and commercial harvest incentive schemes, and some fishing methods. Harvesting is more suitable as a control strategy or for local depletion rather than for eradication (Pasko & Goldberg 2014). It can represent an opportunity to support ecosystem and natural resource management, but it can also incentivise intentional spread of the marine pest.

To reduce populations, harvesting (targeted or commercial) may be suggested to reduce numbers of some invasive marine bivalves, particularly for species with commercial value. For instance, *Perna* spp. are valuable commercial species in their native range and *M. gigas* dominates global aquaculture production and is farmed extensively throughout Australia. Recreational and commercial harvesting can be a potential management option, but it is important to disincentivise movement of animals outside the area of infestation through education, regulation, and enforcement. It's also important to consider water conditions to prevent harvesting of bivalves that may impact human health, such as where there is a risk of paralytic shellfish poisoning.

Recreational and commercial harvest incentive schemes

Any consideration of recreational or commercial harvest must bear in mind that often harvesters may aim to maintain stocks rather than reduce them to non-viable levels, which may not be consistent with management aims. Additionally, transfer of valued species (juveniles or adult stock) to new areas is common and difficult to manage so this must be considered. Invasive marine bivalves that are recreationally harvested for food may become socially or culturally important which has the potential to impair eradication aims. Acceptability as a food source may be high so implications for food safety may also need to be considered.

Incentive schemes may be offered in several ways (Pasko & Goldberg 2014):

- contract operation (commercial):
  - payment to a service provider for the removal or harvest of the invasive marine bivalve
- commercial market (commercial):
  - effort undertaken, usually privately, to harvest and sell the invasive marine bivalve when a perceived market exists
- recreational harvest (recreational):
  - encouragement of recreational fishing of the invasive marine bivalve.

Incentive schemes do not necessarily rely on the marine pest being marketable, although more attractive species require less additional incentive for capture. Many pests have existing markets, and these species may be viewed as a potential resource in their introduced range (Andreakis & Schaffelke 2012). Where incentives are offered, the value of these may need to increase as the pest population decreases to reflect the additional effort required to capture rare individuals (Pasko & Goldberg 2014). To determine if commercial harvest of an invasive marine bivalve is viable, data are needed on catchability, cost of fishing methods, and product value (St-Hilaire et al. 2016). Production of by products such as compost or fertiliser may be viable options for large quantities of product with fewer concerns about degradation.

Community assistance programs

Community assistance in removal of highly abundant marine pest species can increase awareness and generally reduces pest numbers in the short term. There is however the potential for bycatch of misidentified non-target species, and sustained pressure needs to be maintained at appropriate times. Despite this, community assistance programs have been successful during marine pest responses, such as the eradication of the northern Pacific seastar, *Asterias amurensis,* from Inverloch, Victoria, in 2004-05 (Holliday 2005).

5.3.1.4 Benthic sampling and removal

Epibenthic sleds and dredge tows effectively sample benthic assemblages over large areas (Photo 3). Epibenthic sleds and dredges are most effective at sampling infaunal bivalves such as *Potamocorbula amurensis* and *Varicorbula gibba* but have also been successful at sampling subtidal epifaunal bivalves present in soft-sediment habitats, such as *Perna* spp. (Hopkins et al. 2011). A commercial scallop dredge (2.4 metre width) was used to sample for *P. perna* in New Zealand following its detection (Hopkins et al. 2011).

Sled and dredge catch efficiency can be affected by operational factors such as speed of towing, fullness of catch, depth, and substrata. It may be necessary to determine sled and dredge efficiency to help inform survey design (Hopkins et al. 2011). Benthic sampling will be unsuitable for reef habitats or other complex structures and will not work on removing epifaunal bivalves attached to hard surfaces. Epibenthic sleds and dredges can be used to augment other sampling regimes but are not recommended as the sole method of containment, control, or eradication.

Photo 3 Epibenthic sled and use of sled underwater

Source: Chris Woods, NIWA

5.3.1.5 Underwater vacuum, suction, and filtering systems

Underwater vacuum systems are flexible suction hoses attached to small dredges to suck the target organism from marine sediments or from fouling surfaces. Care must be taken to properly filter the water and capture all material to prevent the spread by fragments or release of any larvae (Coutts 2002). Underwater vacuuming is best suited to infrastructure or sites where substrates are primarily sandy.

Use of this method is not suitable for seabeds as poor visibility can be caused by the diver's contact with the seabed, the dragging of the vacuum pipe, and the reverse flushing action used to clear blockages. When used in fine, muddy sediment or where there is a large quantity of biofouling, vacuum filters are easily clogged. Due to the labour-intensive nature and thus high cost of the procedure, diver assisted underwater vacuum is most effective against small infestations.

5.3.1.6 High-pressure water blasting

High-pressure water blasting on land is a cost-effective and an environmentally acceptable method of treating biofouling on infrastructure and should remove all mobile biofouling species (Inglis et al. 2013). High-pressure (>2000 psi for 2 seconds at 100 mm distance) may be required to dislodge biofouling from fissures and crevices. Water blasting has been used to remove established populations of mussels, macrophytes, and tunicates from vessel hulls or other hard substrata, as well as from infected aquaculture equipment. High-pressure water blasting can clean a wide variety of structures. Water blasting could promote release of gametes, so high-pressure cleaning is best combined with additional treatments such as chemical treatment, heat, or desiccation.

High-pressure water sprays typically require treated areas to be either intertidal or removed from the water. *In situ* cleaning by underwater blasting should not be considered for an incursion response unless all viable biological material can be collected.

5.3.1.7 UV light treatment

The application of ultraviolet light (UVC; 100–280 nm) can prevent recruitment on vessel hull coatings and reduce biofouling settlement on reverse osmosis membranes (Hunsucker et al. 2019; Rho et al. 2022). UVC is the most germicidal wavelength in the UV spectrum, and it breaks chemical bonds between DNA and RNA polymers in microorganisms (Braga et al. 2020). This treatment has the potential to cover small and large areas depending on lamp size and transmission intensity. Effectiveness of treatment will be dependent on the light's power, exposure time, frequency of treatment, distance from treatment area, and water quality for light penetration (Hunsucker et al. 2019). Vessel hull construction material and anti-fouling coatings need to be considered as long-term exposure with UVC light has been shown to damage copper coatings (Hunsucker et al. 2019). UV light treatment on invasive marine bivalves has not been specifically tested.

5.3.1.8 Thermal treatment

The efficacy of any thermal treatment is dependent on the susceptibility of the target bivalve's life stages and the ability to maintain the required temperature to achieve mortality. The mass of fouling bivalves and exposure time will need to be considered when planning a treatment. Thermal treatment has low selectivity, but impacts are localised and there are no residues. It is most suitable as a management tool against biofouling, microscopic life-stages, soft-bodied organisms, and species with thin shells such as dreissenid mussel species (Cvetkovic et al. 2015). Complex topographies,

heavy fouling, or taxa with thicker shells such as corbiculid species may require higher temperatures and/or longer exposure times (Inglis et al. 2013).

Thermal treatment may be applied as elevated temperature via:

- hot water
- steam
- created by:
  - electrical elements
  - hydrodynamic cavitation
  - heat torches.

Generally, heat treatment is a favourable treatment option because of its efficacy and low risk to the environment and operations. Bivalves, as hard-shelled organisms, require hotter temperatures (50 to 70°C) for effective mortality than shell-less organisms. The use of heated water between 50 to 60°C can, however, render bivalves non-viable in under two hours (Cahill et al. 2019a; Growcott, Kluza & Georgiades 2016).

Thermal treatment may also be applied as reduced temperature via cold or ambient water to materials and equipment in containment. Cold treatment using supercooled brine has been used to selectively kill pests, or freezing equipment applied as a method of decontamination. Exposure to supercooled brine (–12 to –16°C with 180 to 200 mg/L NaCl) for 60 seconds resulted in 100% mortality of *M. gigas* (Cox et al. 2012).

Thermal treatment of vessels, aquaculture stock, and equipment is an effective treatment method on a small scale. For example, thermal stress has been successfully used to control *P. viridis* (Rajagopal et al. 1995). Exposure of 2 mm of *P. viridis* shells to 39°C water resulted in 50% mortality in 58 minutes and 100% in 73 minutes. Mortality was strongly dependent on age and size of the mussels, with young mussels being more susceptible to treatment than older ones.

Specialised equipment has been developed to contain small areas (1 to 10 m^2) for hot water temperatures (Cahill et al. 2021; Wotton & Hewitt 2004). A wooden ‘hot-water box’ containing heating elements supplied with electricity from a surface support vessel was placed against the target surface by divers and foam seals around the edges prevent exchange of water with the ambient environment.

Substrates that can be removed from the water can be immersed in hot water, or heated water can be applied to contained areas such as niche spaces and piping (Forrest & Blakemore 2006). Heat produced by vessel engines or hydrodynamic cavitation can be used to treat ballast water or vessel internal niches (Leach 2011; Quilez-Badia et al. 2008).

Underwater flame torches cause rapid (<30 s) mortality in clams but substrates need to be considered as infaunal clams buried in mud required heating for up to 5 minutes (Coughlan 2019). Flame torches have been used to destroy intertidal *M. gigas* in South Australia and were deemed suitable for small-scale destruction with added benefit of killing OsHV-1 µvar in oyster tissues. Powerful flame torches may, however, be deemed unsafe and risk damage to infrastructure and handlers.

5.3.1.9 Desiccation and water level manipulation

Desiccation involves the removal of bivalves from the water and exposing them to air to induce drying. Sunlight in combination with desiccation is extremely effective as a general disinfectant, however desiccation is unlikely to be an effective treatment method for bivalves overall. Consideration needs to be given to the life habit of the target bivalve (e.g. intertidal or subtidal) when considering desiccation as a treatment method. Desiccation is only practical where removal of fouled surfaces from the water for an extended period is possible.

Marine bivalves typically have a high tolerance for air exposure and desiccation, particularly intertidal species and thicker-shelled bivalves such as *M. gigas* and *Perna* spp. (Hopkins et al. 2015). However, some bivalves with thinner shells such as *M. sallei* and *A. senhousia* may be more susceptible to desiccation. The recommended length of time required for equipment to be fully dried to ensure all biofouling is killed will be around 21 days (Hilliard et al. 2006). In bivalves, the required length of treatment can vary. For example, adult *M. gigas* require between 16 to 34 days to achieve 100% mortality from desiccation. The air temperature and amount of relative humidity will also affect treatment length. *Perna perna* tolerated air exposure for around 18 days at 15°C and high relative humidity, whereas at 25°C and low relative humidity the mussels survived air exposure for around 1 day (Hicks & McMahon 2003).

Lowering water levels in a water body can cause mortality of submerged organisms through desiccation. Water level manipulation may not be a suitable treatment method for invasive marine bivalves due to general bivalve tolerance to desiccation. The practicalities associated with manipulating water bodies or removing infested structures from the water will need to be considered. Application of these techniques may be restricted to structures that can be removed from the water, or to contained areas where draining of water (drawdown) is feasible.

5.3.1.10 Wrapping and encapsulation

Wrapping and encapsulation uses materials to cover or ‘wrap’ a submerged structure and create an anoxic environment between the wrap and substrate. The wrap creates a watertight barrier, reducing dissolved oxygen levels (anoxia), light, and a potential source of nutrients to accelerate the death of encapsulated biofouling. Invasive marine bivalves will be deprived of light and food, with continued respiration and decomposition of organisms within the barrier depleting oxygen to lethal levels.

The effectiveness of wrapping is improved by the following:

- smothering material is applied continuously without gaps, breaks, or tears to prevent escape of fragments or larvae or ingress of clean water
- use in sheltered environments with low currents because strong currents can make deploying the wrap difficult and increase the risk of tearing
- addition of biocides.

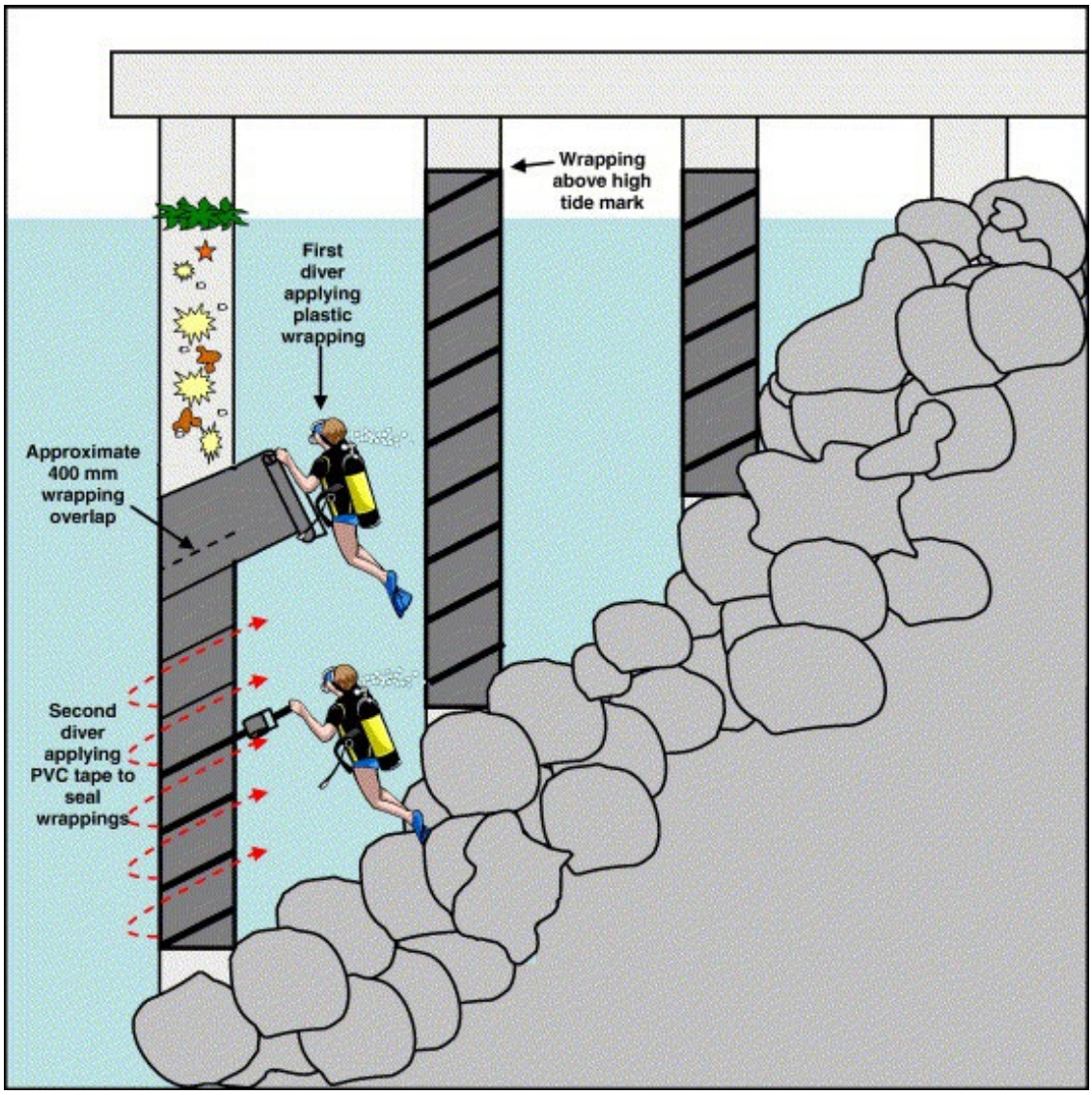
Wrapping and encapsulation of the submerged surfaces of vessels using impermeable barriers, such as polyethylene plastic, have been used to treat fouling on vessels up to 113 metres long (Mitchell 2007). Encapsulation can be used at moderate scales, such as wharf piles (Coutts & Forrest 2005) and other large structures like pontoons. The ability for bivalves to survive hypoxic environments for about a month (Atalah et al. 2016) means that encapsulation is unlikely to be effective as a

treatment method alone. Bivalves are naturally exposed to hypoxic conditions as part of their normal life history. For instance, intertidal bivalves seal themselves off when above the tide line (Riedel, Zuschin & Stachowitsch 2012), and the use of encapsulation or smothering methods on benthic species for extended periods increases risk of damage to the enclosure material. The addition of biocidal chemicals such as chlorine or acetic acid may expedite treatment.

For vessels, polyethylene silage plastic wrap (125 µm thick) can be cut to size to suit the vessel type and is deployed by divers in association with a topside support team (Mitchell 2007). The plastic is passed from one side of the vessel to the other, overlapped and secured tightly using PVC tape or ropes to create a dark watertight environment. Commercial encapsulation tools, such as FAB Dock, are available which can be applied to a vessel arriving in port, or to a vessel at anchor, alongside a wharf or in a marina berth. Commercially available floating boat docks up to 30 m have been shown to be useful for emergency treatment of biofouling on small vessels. Wrapping of vessels >25 metres is labour intensive and may take up to two days to deploy. The time needed for effective treatment is around 7 days, which may be too long when rapid treatment and vessel turnaround time is crucial. Wraps on wharfs can also be damaged by berthed vessels (Coutts & Forrest 2007).

During an ascidian incursion in New Zealand, divers encapsulated wharf piles using sheets of polyethylene silage plastic wrap (125 µm thick) and rolls of black polyethylene (1 m wide × 50 µm thick) by wrapping around the piles in a circular motion overlapping each successive wrap by ~400 mm (Figure 7; Coutts & Forrest 2007). Sharp objects on the hull or pylon, such as propeller blades, oysters, or fixings, should be wrapped separately or covered with tubing or cloth before encapsulation to prevent tears in the plastic.

Figure 7 A schematic of the polyethylene wrapping method used to treat wharf piles



Source: Coutts & Forrest (2007)

If properly deployed, the wrap should contain the pest species and its larvae. Extreme care should be taken to ensure that biofouling is not dislodged when the wrap is deployed. Generally, the wrap must remain in place for at least seven days if no biocide is used to achieve the desired effect, and likely longer for invasive marine bivalves (Inglis, Floerl & Woods 2012).

Wrappings can themselves be colonised by biofouling species (Coutts & Forrest 2007). Encapsulation is not effective if substrates underneath structures such as pilings are not also treated, and therefore encapsulation should be complemented with smothering and other treatments (Coutts & Forrest 2007; Deibel et al. 2014).Wrapping also produces large amounts of plastic waste. This waste must be disposed of in landfill or an approved solid waste treatment facility. Consideration also needs to be given for any protected species that could be impacted by wrapping, such as EPBC Act listed species. Relevant state or territory agencies should be consulted about the suitability of wrapping and encapsulation method for a vessel or structure.

5.3.1.11 Smothering

Like wrapping and encapsulation, smothering benthic habitats by covering them with plastic, geotextile fabric, or burial with sediment (such as dredge spoil) can effectively treat relatively localised infestations. Smothering:

- Has low selectivity, but impacts are localised
- leaves no residues
- is applicable to sessile or sedentary species (on surfaces that can be covered) and benthic species
- is relatively affordable.

The material used to smother the surface must be continuous, without gaps or breaks in material to avoid escape of larvae. Tolerances to burial by sediment is variable between marine pests and some can tolerate prolonged periods (>2 weeks) of burial (Glasby, Creese & Gibson 2005). Smothering as a control method has not been specifically tested on invasive marine bivalves. However, gas impermeable benthic barriers have demonstrated suppression of invasive freshwater bivalves in the USA, including the zebra mussel, *Dreissena polymorpha,* and the freshwater gold clam, *Corbicula fluminea* (Conry et al. 2024; Wittmann et al. 2012). Conry et al. (2024) installed a polyvinyl chloride benthic barrier covering an area of ~3,900 m^2 on the shoreline and littoral zone of a freshwater lake in Lake Waco, Texas. The barrier was left in place for five months with frequent monitoring, and most *D. polymorpha* were killed by the barrier with lasting effects over five years, but the treatment did not achieve 100% mortality. These studies are on freshwater bivalves in enclosed freshwater lakes, so the effects of smothering on marine bivalves in marine environments may not show the same results.

Anaerobic mats may be used as a treatment for benthic organisms and can have similar effects to wrapping. Jute mats have been used to cover and kill the invasive algae *Caulerpa taxifolia* in New South Wales and subsequently killed all other marine benthic organisms (Creese, Davis & Glasby 2004).

The application of smother material to be used is dependent on the seabed or substrate. For example, smothering of flat or gently sloping soft-sediment seabeds with uncontaminated dredge spoil had varying effects on *D. vexillum* in New Zealand (Coutts 2006; Coutts & Forrest 2007). Geotextile fabric sheets proved to be more suitable for steep gradient rip-rap seabeds as geotextile sheets are unable to hold dredge spoil.

5.3.2 Chemical treatment

The dynamic nature of marine environments means that any biocides or chemical agents, such as chlorine, salt, or pesticides released into them are rapidly diluted and dispersed. This is problematic when the agent must be above a threshold level to be lethal. Very high concentrations may need to be released or the area may need to be enclosed for the treatment to be effective (Anderson 2005; Ferguson 2000). Conversely, where the agent is effective at very low concentrations, rapid dispersion by water may achieve broad dispersal.

Continuous exposure to chemical treatments forces the closure of the bivalve shells cutting off oxygen and food sources. Bivalves can withstand functioning under anaerobic conditions for a period

of time. Intermittent exposure to chemical treatment is therefore unlikely to be effective and continuous treatment is required.

The major considerations for the use of chemical treatments in water bodies include the following:

- volume of water that needs to be treated (a function of the area, depth, and degree of flushing of the waterway)
- presence, susceptibility, and value of non-target organisms that may also be affected
- water quality (e.g. organic matter may consume or bind some chemicals)
- residual effects of any toxicants on the surrounding environment and human health
- safety management when handling large volumes of chemicals.

Incident managers should consider the use of chemical control in aquaculture and the potential for negative effects on future marketability of a product or useability of the infrastructure, e.g. copper compounds may inhibit phytoplankton production in ponds.

An extensive range of chemicals have been trialled in the laboratory for their efficacy against marine pests (McEnnulty et al. 2001). Several effective chemicals for invasive marine bivalves are presented below in more detail.

Chemicals that have been evaluated for their efficacy against various marine organisms comprise two forms:

- oxidising biocides and agents:
  - chlorine (gas, sodium or calcium hypochlorite, or chlorine dioxide)
  - bromine and organobromines
  - active halogen compounds
  - ozone
  - hydrogen peroxide
  - mild acids (such as acetic acid)
- non-oxidising biocides (Jenner et al. 1998):
  - aldehydes
  - amines
  - organometals
  - brine or lime
- Detergents and disinfectants
  - quaternary ammonium compounds.

5.3.2.1 Permits to use chemical treatments

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is the Commonwealth authority responsible for the assessment and registration of all agricultural and veterinary chemical products in the Australian marketplace. The APVMA contains a list of all approved chemical products that are available in Australia and can be found via the APVMA PubCRIS database search webpage. Any required variations to be made to these chemicals must be approved by APVMA.

In most states and territories, registered chemical products must only be used for the purposes specified on the label. Any use of chemicals for the control of invasive marine bivalves is likely to differ from that specified on the label. In these cases, permits need to be sought from APVMA to use chemicals in a different way. APVMA can also consider applications for permits allowing limited use of an unregistered chemical product. The time it will take for a permit to be issued will depend on the nature of the application, existing risks, and whether the APVMA needs to seek additional information. For emergency use permits, it can take up to two weeks for permits to be processed, while minor use permits can take up to three months.

In addition to seeking APVMA approval for use of chemicals to control invasive marine bivalves, there will often be other stakeholders who need to be consulted and consent to chemical use, such as port authorities, local governments, environmental government agencies, and national park managers.

5.3.2.2 Industrial detergent, disinfectant, or de-scaler

Disinfectants and detergents are generic terms used to describe chemical agents or formulations designed to kill microorganisms on surfaces. Quaternary ammonium compounds are found in a variety of cleaning and disinfection productions and have been trialled against invasive marine bivalves (Cahill et al. 2021). Commercial marine detergents, disinfectants, and de-scalers, such as Conquest®, Quatsan® or Rydlyme®, respectively, deteriorate and/or dissolve biofouling and are biodegradable.

Conquest® is a highly effective detergent and Quatsan® is highly effective disinfectant that can cause 100% mortality of the fouling mussels *Mytilus galloprovincialis* within 14 hours at concentrations of 1% and above (Lewis & Dimas 2007). Similar dosages could be effective against other mytilid mussels like *Mytella strigata.* However, 100 ppt Quatsan® for up to 12 hours was ineffective against adult *Saccostrea glomerata* (Neil & Stafford 2005), suggesting that stronger concentrations may be needed for more resilient bivalves or is an ineffective treatment option for them. Specific detergent regimes will be needed before treating an invasive marine bivalve.

Rydlyme® at 25% concentration for 14 hours is the recommended application time to dissolve significant mussel growth (Lewis & Dimas 2007). A linear relationship between the level of fouling and the volume of Rydlyme® required to digest fouling has been developed for this treatment (Lewis & Dimas 2007). Rydlyme® may dissolve growth in this period, whereas other preparations may weaken it but not dissolve it.

Consultation with vessel or infrastructure owners needs to be considered as some of these preparations have been associated with damage to internal seawater systems. Toxicity of detergents, disinfectants, and de-scalers need to be considered prior to use.

5.3.2.3 Chlorine

Chlorination is the most common form of chemical treatment used in enclosed water systems because of its cost, availability, and wide-spectrum efficacy. Chlorine breaks down naturally and has minimal long-term effects on the environment. Exposure to light, elevated temperatures, and reaction with organic compounds in the water accelerates the reduction in chlorine concentration. For this reason, it is important to monitor levels of 'free available chlorine' in the treated area, as often as every fifteen to thirty minutes initially.

Chlorination does have some inherent problems associated with its use:

- impacts on non-target organisms
- non-uniform distribution of residual chlorine (Rajagopal et al. 2003a; Rajagopal et al. 2003b; Rajagopal et al. 2006)
- hazards of handling chlorine gas cylinders or concentrated chlorine solution
- difficulty in maintaining chlorination plants in the operational area.

Morrisey et al. (2016) recommended that a dose of 200 ppm chlorine for at least four hours is effective against invasive marine bivalves. This dose and length of time was effective against *M. gigas* and may be for other bivalves. However, in *Mytilopsis sallei,* longer chlorine treatments may be needed because four hours was not effective (Bax et al. 2002).

Chlorine was used to assist with eradication of *M. sallei* after its incursion into three locked marinas in Darwin, Northern Territory (Ferguson 2000). The entire 600 million litres of sea water in the 12-hectare Cullen Bay Marina and Lock in Darwin were treated with liquid sodium hypochlorite. Chlorine was selected because it had been successfully used to control a related mussel, *Dreissena polymorpha,* in the USA (Bax et al. 2002). Several thousand tonnes of sodium hypochlorite were added directly to Cullen Bay Marina to achieve a concentration of 10 mg/L. Large pumps were used to control chlorine dispersion and avoid stratification. Laboratory trials indicated that mortality was likely if the concentration of chlorine could be increased to 24 mg/L and maintained for >90 hours (Bax et al. 2002). A concentration this high for that long was unachievable because of the volume of water that required treating. Therefore, powdered copper sulphate (equivalent to 0.5 mg/L in solution) was subsequently used in conjunction with the sodium hypochlorite in the marina to kill the mussels. The treatment regime was used for six days, and the mussels were eliminated successfully.

Although successful in eradicating *M. sallei*, the high concentration of chlorine used for that length of time killed all marine organisms in the marina and demonstrates potential ecological impacts. However, this was balanced against the potential negative impact *M. sallei* could have on the environment, fisheries, and marine infrastructure if the invasive mussel population were to become established (Summerson et al. 2013).

5.3.2.4 Acetic acid

Acetic acid has low selectivity and is suitable for immersion and enhancing the effect of desiccation, wrapping, and encapsulating. Immersion at 4% acetic acid (in sea water) for 1 minute removes soft-bodied fouling organisms from shellfish seed stock (Forrest, Hopkins & Gardner 2007). Effectiveness of acetic acid is dependent on concentration and immersion time. Low concentrations of acetic acid

(4%) are equivalent to domestic vinegar and do not represent significant environmental or occupational risks if handled appropriately (Forrest, Hopkins & Gardner 2007). When treating aquaculture stock, it is important to understand the minimum time required to remove the marine pest and minimise stock mortality.

Exposure to 100 ppt acetic acid for 12 hours killed around 75% of the oyster *S. glomerata* (Neil & Stafford 2005). Although *S. glomerata* is native to Australia, the concentration and exposure time used by Neil and Stafford (2005) could be applied to other bivalves, including other oysters such as *M. gigas* and *M. bilineata*. Similar work with acetic acid showed that encapsulation with 40 ppt acetic acid resulted in 100% mortality of the mussel *Semimytilus algosus* within 24 hours (Keanly & Robinson 2020).

5.3.2.5 Copper sulphate

This treatment will be most suited to closed waterways, internal water systems and aquaculture equipment removed from the water. A trial of copper sulphate (Cu 1.5 mg/L) used in combination with chlorine in the infested Cullen Bay Marina, Darwin, resulted in 100% mortality of *M. sallei*. Copper sulphate powder was dissolved in a road construction watering truck tank and hosed over the water surface of the 'mixed' marina (McEnnulty, Jones & Bax 2001). Copper sulphate's low specificity and persistence in the environment should be considered when weighing up treatment options. Copper sulphate can have environmental impacts and may be regulated by legislation or by the waterway managers. Copper may remain in the system and be reactivated when conditions permit, and even low concentrations can affect phytoplankton.

5.3.2.6 BioBullets

A commercial biotechnology company, BioBullets, manufactures encapsulated actives specific for controlling bivalve molluscs. The active chemicals are not supplied; instead, dosing programmes are tailored depending on the target organism and its environment. In principle, BioBullets are encapsulated active compounds designed to mimic natural food items that are taken up by filter feeding bivalves. They are considered safe for use in drinking water facilities, do not bioaccumulate, and degrade within the environment within a few hours of entering the water. Laboratory exposures demonstrated that two formulations effectively controlled the invasive Gulf wedge clam *Rangia cuneata*. A single dose of 2–6 mg/L of the active ingredient in a static system achieved 90% morality after 30 days of exposure (Tang & Aldridge 2019). BioBullets are promising as a treatment option, however, this method remains to be fully assessed under field conditions in the marine environment. It may also be ineffective at treating bivalve populations in large open systems. It could be effective however at controlling populations in closed or semi-closed systems. For instance, Tang and Aldridge (2019) estimate that in order to treat the entire 10.4 km drainage channel occupied by *R. cuneata* in the United Kingdom would require 4.7 tonnes of BioBullets with an active concentration of 10 mg/L. Tang and Aldridge (2019) concluded that this is ecologically and logistically feasible.

5.3.2.7 Osmotic treatment

Osmotic treatment is the manipulation of salinity levels and has been used in several marine pest incursions. Depending on the marine pest's tolerance, exposure to hyposaline (via addition of freshwater) or hypersaline (via addition of salt) conditions can disrupt the osmotic balance resulting in death. It can take the form of immersion of infested structures or equipment in fresh water,

manipulation of salinity in enclosed water bodies through re-diversion of fresh or salt water, or through application of large quantities of salt near the target organism.

Salt is inexpensive, easy to obtain, safe to handle, and can be applied on a large scale with the appropriate resources such as a barge, backhoe, hopper, or diver guidance. This technique becomes less efficient as the area being treated increases or when applied to steep slopes and high-relief habitats (such as rocky reef). Salt treatment is not suitable for application in high-energy environments since salt would be rapidly dispersed by ocean-generated swell. The efficacy depends on absolute salinity change and the rate of change in salinity as well as the species' tolerance. The rate of salinity change is likely to be slow for large treatment volumes, so treatments are likely to be most effective for small, enclosed areas. Whilst application of salt can be effective, it can also be detrimental to other species and should be considered when planning response activities.

The salinity tolerance of a species can vary according to life-stages and may also be affected by other factors such as temperature, nutrient, or oxygen levels. The efficacy of salinity manipulation for invasive marine bivalves will depend on their ability to withstand prolonged exposure to an altered regime.

Osmotic treatments may only be effective on larval life stages of invasive marine bivalves because they typically have a narrower environmental tolerance than adults. Many adult bivalves occupy estuarine systems that frequently naturally experience freshwater pulses, particularly after periods of heavy rain, and have tolerance of a broad range of salinity. *Potamocorbula amurensis* has been demonstrated to tolerate 10 ppt step changes in salinity. Some bivalves such as *M. sallei* have also been recorded in freshwater systems kilometres from the coast (Tan & Morton 2006) rendering hyposaline treatments ineffective.

5.3.2.8 Combined chemical and physical treatment

Some bivalves are especially tolerant of anoxic environments and wrapping and encapsulation alone may not be an effective method to destroy them in a time-efficient way. Speed of effectiveness of wrapping and encapsulation can be improved through the addition of biocides such as chlorine or acetic acid (Ammon et al. 2019). Chlorine is a biocide commonly used in wrapping and encapsulation that is generally used at >200 ppm for at least 24 hours (Ammon et al. 2019). Concentrations must be measured regularly to ensure that active concentration is maintained because active chlorine levels drop dramatically in presence of large amounts of organic matter.

Commercially available floating boat docks up to 30 metres have been shown to be useful for emergency treatment of biofouling on small vessels. The addition of chlorine (e.g. 'dichlor') at an initial concentration of 200 mg/L killed all fouling organisms on a vessel within six days and was effective for 90% of the study's target organisms, with *M. gigas* oyster attached to the hull killed within six days (Morrisey et al. 2016). These types of floating docks could be a good alternative to wrapping for treating small vessels during an emergency response.

5.3.3 Biological and ecological control

Biological and ecological control occurs by the manipulation of environmental conditions to create an adverse habitat for a species' survival and reproduction. They may include the use of natural predators, competitors, parasites, or pathogens to suppress population growth. Biological and ecological control are not a rapid response operation as an extensive and lengthy review process

must occur before a biological control agent can be released into the environment. The introduction of non-native species or exotic disease to effect control is not advised due to the potential issues posed by these additional introductions, especially given that impacts are likely to be irreversible (Giakoumi et al. 2019). Promoting predation or herbivory by native species, or utilising endemic diseases, are more acceptable approaches but could still produce undesirable impacts, and their efficacy is unclear (Smith 2016).

Natural predation on established populations of invasive marine bivalves may be effective but is not amenable to control by response personnel. Astudillo et al. (2018) tested the potential of top-down biocontrol from the predatory whelk *Reishia clavigera* on invasive populations of *M. sallei* in Hong Kong. While *R. clavirgera* preferentially predated on *M. sallei*, this was driven by predation vulnerability and may not be an appropriate management method (Astudillo et al. 2018).

Table 5 Examples of treatment methods used to control some invasive marine bivalves

Bivalve species	Physical treatment	Chemical treatment	Biological/ecological control
Magallana gigas	Exposure to 43°C for 1 hour resulted in 100% mortality of *M. gigas* (Rajagopal et al. 2005). Piola and Hopkins (2012) reported 57.5°C for 60 minutes or 60°C for 30 minutes for 100% mortality of *M. gigas.* Blow/flame torches have been used to help kill wild *M. gigas* during a POMS outbreak in South Australia.	Field application of chlorine to an encapsulated vessel at 200 ppm was effective for *M. gigas* in New Zealand (Morrisey et al. 2016). Acetic acid at 100 ppt killed 75% of a related species *Saccostrea glomerata* within 12 hours (Neil & Stafford 2005). Detergent Quatsan™ at 100 ppt was ineffective against *Saccostrea glomerata* (Neil & Stafford 2005).	Environmental levels of ostreid herpesvirus-1 microvariant (OsHV-1 µvar) have been ineffective at controlling wild populations of *M. gigas* in New South Wales.
Mytella strigata	Hand removal by divers from a vessel (CCIMPE, unpublished).	Conquest (>5 ppm) was applied to a vessel using a cofferdam structure in place for >13 hours to kill molluscs after detection of *M. strigata* (CCIMPE, unpublished). Exposure to >10 ppt Quatsan™ or Conquest™ was effective against adult *Mytilus galloprovincialis* (Lewis & Dimas 2007).	No data currently available
Mytilopsis sallei	Hand removal from vessels and marina infrastructure during localised small-scale incursions (Willan et al. 2000).	Chlorine in combination with copper sulphate was successful in eradicating *M. sallei* from locked marinas in Darwin in 1999 (Bax et al. 2002; Ferguson 2000). Chlorine concentration was >24 mg/L over 6 days. Conquest (>5 ppm) was applied to a vessel using a cofferdam structure in place for >13 hours to kill molluscs after detection of *M. sallei* (CCIMPE, unpublished).	The predatory whelk *Reishia clavigera* preferentially predated on invasive populations of *M. sallei* in Hong Kong. However, this was driven by predation vulnerability (Astudillo et al. 2018).

Bivalve species	Physical treatment	Chemical treatment	Biological/ecological control
Perna spp.	Removal of *P. perna* in New Zealand using a commercial dredge (Hopkins et al. 2011). 2 mm *P. viridis* exposed to 39°C resulted in 50% mortality in 58 minutes and 100% in 73 minutes; mortality was strongly dependent on size and age of mussel. *P. viridis* has been removed from vessels by hand using divers, or with high pressure hoses (CCIMPE, unpublished). *P. canaliculus* was removed by divers from a small, contained infestation in South Australia (CCIMPE, unpublished).	Conquest (>5 ppm) was applied to a vessel's in-water systems for over >10 hours to kill molluscs after detection of *P. viridis* (CCIMPE, unpublished).	No data currently available

6 Decontamination, destruction, and disposal

This section contains material summarised or adapted from the Aquatic Veterinary Emergency Plan (AQUAVETPLAN) manuals because of similarities in decontamination, destruction, and disposal methods suitable for invasive marine bivalves. This section is intended to be used in conjunction with the AQUAVETPLAN manuals which detail methods of disease control:

- decontamination (AQUAVETPLAN – Operational Procedures Manual – Decontamination)
- destruction (AQUAVETPLAN – Operational Procedures Manual – Destruction)
- disposal (AQUAVETPLAN – Operational Procedures Manual – Disposal).

See Section 5.3 for treatment methods that can assist with decontamination and destruction of invasive marine bivalves in addition to the above AQUAVETPLAN manuals.

6.1 Decontamination

Decontamination is the cleaning or treatment of material used to remove invasive marine bivalves or render bivalves non-viable, including their propagules and any parasite and pathogen that can be associated with the marine pest species (Young et al. 2017). Some decontamination occurs *in situ* and no separate disposal activities occur. Other methods, such as most physical removal, require removal and capture of invasive marine bivalves and it is vital that destruction and disposal occur. Appropriate decontamination procedures are required to allow personnel, machinery, and equipment to move safely between locations during response operations.

The decontamination process comprises several stages (DAFF 2022a):

- planning:
  - identification and assessment of risks
  - design of efficient and effective procedures
  - training of personnel
- implementation:
  - cleaning
  - disinfection
  - waste treatment and disposal.

If decontamination is required, a plan should be developed considering the following information:

- the nature of the pest and how is it most effectively removed
- type of environment, material, or equipment requiring decontamination
- water supply quality and quantity:
  - organic matter rapidly inactivates a number of chemical disinfectants

- available options for disinfection:
  - including disinfectant chemical compatibility if multiple agents are in use
- risks to the safety of personnel and equipment:
  - disinfectants can be corrosive, and most are irritants to people
- environmental pollution risks:
  - most disinfectants are toxic to aquatic life, although some are degraded quickly
- relevant legislation or regulations that must be complied with.

Effective cleaning is responsible for more than 90% of the success of decontamination. However, accumulations of soil, dirt, organic matter, or biofouling provide an effective barrier which may protect invasive marine bivalves from disinfecting agents. Wash water may still contain viable gametes or larvae and must be disposed of appropriately. Effectiveness of cleaning compounds and disinfectants will depend on:

- water quality (such as suspended matter) and hardness
- concentration and contact time
- temperature and pH.

6.2 Destruction

Destruction occurs to aid in disposal of a captured invasive marine bivalve or to control the spread of disease (in case of disease management) via methods employed during containment, control, or eradication efforts. For example, destruction may be required after the collection of vessel fouling material, aquaculture, stock, or equipment. However, destruction of stock or equipment may not always be required since removal from water will ultimately result in death for the invasive marine bivalve (see Section 5.3.1.9). In aquaculture contexts, tolerance to desiccation should be considered for both the bivalve and the farmed aquaculture stock prior to removal from the water. Treatment for closely related marine pest and stock species may result in the death of the stock. For example, an invasive marine bivalve and cultured bivalve stock may have similar desiccation tolerance and be destroyed at the same time when removed from the water.

Invasive marine bivalves may be destroyed *in situ* or removed and destroyed elsewhere. The timing of mortality is variable among taxa, and exposure to air will result in stress to the organism. The time between removal of the invasive marine bivalve from the water and destruction should be as short as practically possible. This will minimise the organism's stress and the risk of escape.

Where invasive marine bivalves are removed and destroyed elsewhere, the site used for destruction should be contained to prevent release of the bivalve, viable propagules, or pathogens and parasites. Ideally the destruction site should be close to the area from which bivalves are being removed, and/or to the disposal site. Appropriate disposal sites and methods should be identified prior to commencing destruction activities. Due to the volumes of fluid associated with destroying invasive marine bivalves, surface or groundwater contamination and seepage back into marine environments must be managed carefully.

Destruction plans should consider the following (DAFF 2009):

- if destruction will occur *in situ* or at another location
- the volume and type of invasive marine bivalve to be destroyed
- how the bivalve will be contained until destruction
- any pathogens or parasites carried by the bivalve that will also need to be destroyed
- facilities and equipment available for destruction method
- appropriate destruction methods (see AQUAVETPLAN – Operational Procedures Manual – Destruction)
- potential environmental and human health impacts and any relevant legislation (e.g. chemical use and dead biomass)
- any required decontamination and disposal method
- any permits required by authorities for dealing with species listed in legislation.

Information pertaining to ethical concerns, which will depend partly on legislative and legal requirements of the jurisdictions involved, can be found in the following resources:

- Australian Animal Welfare Strategy (AAWS) (currently being reviewed)
- Australian code for the care and use of animals for scientific purposes.

6.3 Disposal

The primary reasons for disposing of a marine pest, their products, materials, and waste is to remove or deactivate the marine pest's reproductive, regenerative, or disease transmission potential. Disposal should be completed as soon as possible after capture or destruction. Disposal has social, environmental, and aesthetic impacts that need to be considered.

Several considerations for a disposal plan for marine pest waste are summarised below (DAFF 2022b):

- selection of disposal site and transport to the disposal site
- method of disposal
- items that may require special consideration (e.g. liquid waste, control of scavengers)
- media and community communication.

Appropriate arrangements are required for the disposal of invasive marine bivalve waste. A decision-making framework developed for identifying appropriate disposal has been developed (AQUAVETPLAN – Operational Procedures Manual – Disposal). In summary, an incident manager should consider the following:

- is the method consistent with international agreements and standards?
- are acceptable transport methods available?

- does the method meet legislative requirements, and can the necessary regulatory approvals be obtained?
- is the method consistent with industry standards and agreements?
- is the method cost-effective?
- how quickly will the method resolve the disposal problem?

Appendix A: Taxon-specific information on some invasive marine bivalves to Australia

*Note – Information in this Appendix was up to date as of February 2025.

Family Corbulidae

Potamocorbula amurensis

Potamocorbula amurensis is known as the Asian basket clam, the Amur River clam, the Asian brackish water clam, or the overbite clam. It is not recorded in Australia. *Potamocorbula amurensis* is native to Russia and China and was introduced to San Francisco, USA, in 1986. In 2020 it was recorded in Belgium. *Potamocorbula amurensis* is a small clam with a shell length up to 25 mm. The clam is known for its broad salinity tolerance and high survival rates in adverse conditions. The environmental tolerance of this clam is suspected to have played a major role in its successful trans-Pacific spread from the northwest Pacific to San Francisco. *Potamocorbula amurensis* can create very high densities, >10,000 individuals per m^2, which can negatively impact native fauna by outcompeting them for space and food.

Potamocorbula amurensis is nationally listed in Australia on the Exotic Environmental Pest List (EEPL) and is also listed on the top 100 worst invaders list.

Table 6 Taxonomic classification of *Potamocorbula amurensis*

Classification	*Potamocorbula amurensis*
Phylum	Mollusca
Class	Bivalvia
Order	Myida
Family	Corbulidae
Genus	*Potamocorbula*

Diagnostic features for identification

Field identification

Potamocorbula amurensis cannot be identified in the field with a high degree of taxonomic certainty (MPSC 2018).

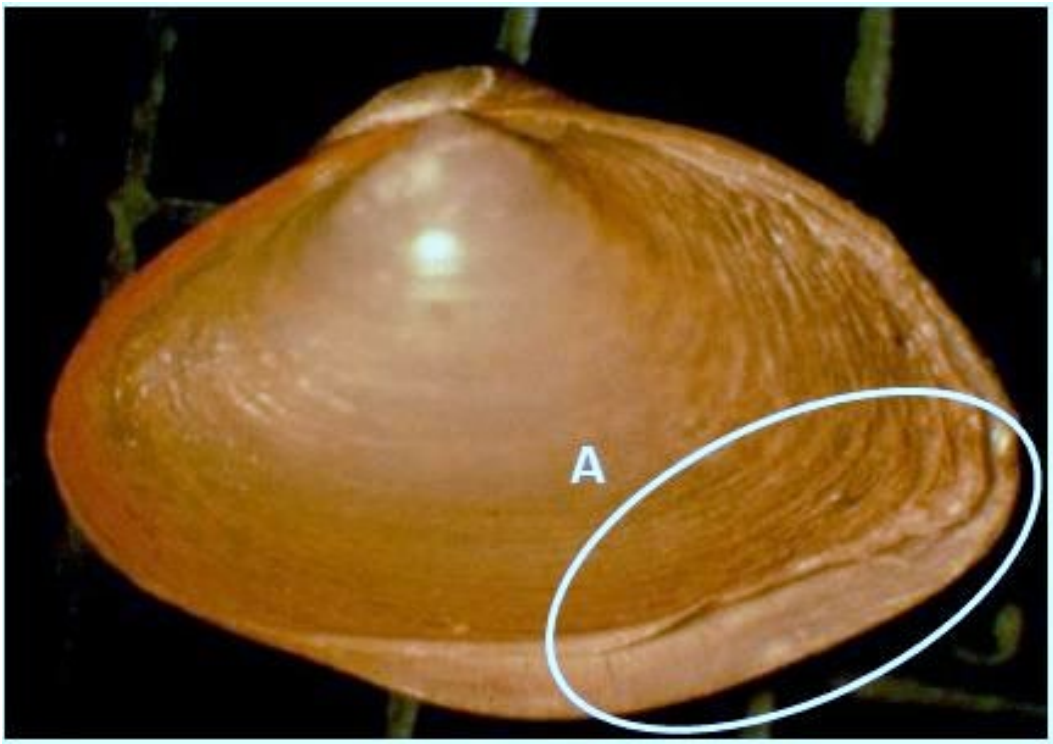
The shell of *P. amurensis* is ovate and thin and can measure up to 25 mm in length. Colour of the shell can be yellow, tan, or off-white with brown staining (Photo 4). The shell surface is usually smooth but can be wrinkled in older individuals.

The right valve is decidedly larger than the left valve (hence the name, overbite clam). The right valve has a narrow tooth which is attached to the shell wall below the hinge line. The left valve has a long, projecting chondrophone (corresponding 'socket' to the right valve tooth). Pallial line with a

small posterior sinus. Beaks anterior to midline; anterior end and posterior end are both sharply rounded. An exterior posterior keel is apparent on the left valve. The hinge plate is narrow.

Characteristic of this clam is the distinctive shell 'overbite', where one shell is much larger than the other (Photo 4 and Photo 5). The overbite can be around a quarter to a third of the total shell perimeter edge.

Photo 4 Adult *Potamocorbula amurensis* showing the shell 'overbite' (top), and the inside and outside of the shells (bottom)



A Shell 'overbite' where one shell valve is much larger than the other.
Source: Janet Thompson, United States Geological Survey, California.

Photo 5 Example of inside and outside shell valves of *Potamocorbula amurensis*

Source: Evan Rees, Northern Australia Quarantine Strategy (NAQS)

Similar native species

Potamocorbula amurensis is unlikely to be distinguished from native Australian bivalves in the field. It is morphologically very similar to native Australian Corbulidae (i.e. *Corbula* (*Serrocorbula*) *verconis*), Myidae (genus *Cryptomya*), Mesodesmatidae (genus *Paphies*), Tellinidae (genus *Tellina*), and some Mactridae spp. (notably the genus *Spisula*). It also shares morphological similarities to the introduced *Varicorbula gibba* (MPSC 2018, Richard Willan [MAGNT], pers. comm., April 2023).

Photographs of some similar native bivalves to *P. amurensis* can be found at the marine pests website.

Laboratory and molecular identification

A qPCR assay targeting the 18S rDNA gene of *P. amurensis* has been developed for species-specific detection from environmental samples (Smith et al. 2012). *Potamocorbula amurensis* larvae were spiked into sample matrices including benthic assemblages, biofilms, sediment grabs, and plankton net hauls. The qPCR had a limit of detection of one larva in 10 g of sediment and five larvae in 10 g of benthic invertebrate and macro-algal assemblages (Smith et al. 2012). This qPCR has not been validated under Australian conditions and this needs to be considered before using this method.

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Potamocorbula amurensis is an infaunal clam. In its introduced range of San Francisco, USA, it has not been observed as epifaunal. *Potamocorbula amurensis* prefers the lower-intertidal and subtidal and typically is observed between 1 and 17 m water depth. The clam can inhabit any sediment type, including silt, sand, gravel, mud, shell sand/grit, and hard packed clay. In its native range in the

Yangtze River estuary, China, *P. amurensis* is frequently found in fluid mud, highlighting that sediment type does not limit its distribution (Chao et al. 2012).

The water temperature range for *P. amurensis* is between 5 and 25°C (Thompson & Parchaso 2012) and the salinity range is between 0.1 ppt and 33 ppt. Nicolini and Penry (2000) showed that adult *P. amurensis* can tolerate weeks of exposure at 35 ppt.

Potamocorbula amurensis is an efficient suspension feeder with a versatile diet (Parchaso & Thompson 2002). The versatile diet is suspected to be a key to its invasion success, being able to feed on food of various sizes (Parchaso & Thompson 2002). *Potamocorbula amurensis* can consume large amounts of zooplankton when at high densities, reducing food sources for other marine organisms. When at high densities, *P. amurensis* have outcompeted several bivalves, disrupting established ecological communities in the San Francisco Estuary. It should be noted that San Francisco Estuary is heavily polluted, and this may influence the competitive interactions that *P. amurensis* has with ecological communities in the estuary. Baumsteiger et al. (2017) found that salinity is a strong driver in the abundance and distribution of *P. amurensis* in San Francisco Estuary.

Predators of *P. amurensis* will likely include any benthic feeding fish capable of consuming the clam. For example, sturgeons are the main predator of *P. amurensis* in its native range. Other predators include sea birds and crabs.

Reproduction and growth

Potamocorbula amurensis has separate sexes. Usually, *P. amurensis* spawns twice a year, typically around summer and autumn, however, field studies indicate that *P. amurensis* continually trickle spawn throughout the year (Parchaso & Thompson 2002). Spawning can occur in a broad range of salinities and water temperatures. In its introduced range, *P. amurensis* has been observed spawning between 6 and 23°C water temperature and salinity between 0.1 and 27.6 ppt (Parchaso & Thompson 2002). Females release between 45,000 and 220,000 eggs which are non-buoyant and are fertilised near the sediment. Fertilised eggs develop into non-mobile and non-feeding trochophore larvae within 24 hours of fertilisation. Feeding veliger larvae develop 7 to 24 hours later where they continue to develop in the water column until they settle between days 17 and 19. Newly settled clams can become reproductively mature within two months, which is around 5 mm shell length. *Potamocorbula amurensis* can live for around 2 to 2.5 years (Baumsteiger et al. 2017).

Gametes of *P. amurensis* are highly tolerant of changes in salinities, capable of surviving 10 ppt step increase or decrease in salinity. Embryos that are two hours old can tolerate salinities from 10 to 30 ppt and by the time they are 24 hours old they can tolerate the same range of salinities (2 to 30 ppt) as adults (Nicolini & Penry 2000).

Pathways and vectors

Potamocorbula amurensis was introduced into San Francisco Estuary from its native range by ballast water (Carlton et al. 1990). All life stages of *P. amurensis* can tolerate a broad range of water temperature and salinity increasing its chance of establishing once discharged into a new area. *Potamocorbula amurensis* is not an epifaunal species, so is unlikely to spread via biofouling. However, Davidson et al. (2008) recorded *P. amurensis* within sediment accumulated within a biofouling community on a vessel. Once introduced to an area, *P. amurensis* can disperse throughout an estuarine system. Newly settled and juvenile clams can disperse attached to other particles or

surfaces that can drift. This is unlikely to result in large geographic range extensions but could be an important secondary pathway.

Potential impacts

High densities of *P. amurensis* can outcompete native species for space and food. A fivefold decrease in phytoplankton production in San Francisco Estuary has been attributed to high density *P. amurensis* populations. Two copepods, a rotifer, and a mysid shrimp concurrently declined with the phytoplankton, presumably due to food limitation or direct consumption by *P. amurensis* (Kimmerer et al. 1994).

Sousa et al. (2009) have also proposed that *P. amurensis* can indirectly alter the ecosystem once introduced by changing sediment chemistry, light penetration in the water column, sediment type, and hydrodynamics. High density populations can also impact fisheries, by interfering with gear as bycatch or by limiting target fisheries species by taking up their space.

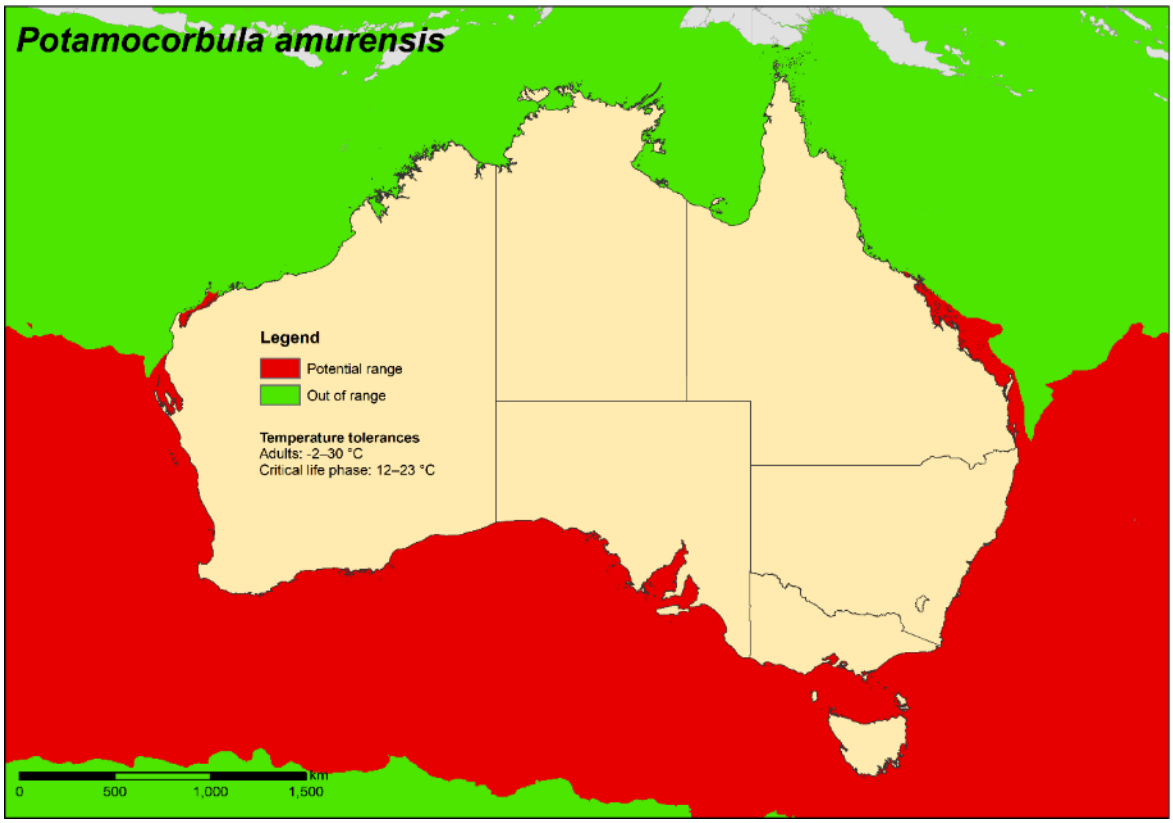
Global and Australian distribution

Potamocorbula amurensis is native to the estuarine habitats of Russia to southern China. It has been introduced to San Francisco Bay, USA, in 1986 where it has become established and has caused negative impacts on the benthic community (Carlton et al. 1990). In 2020, *P. amurensis* was reported from Europe for the first time, when it was detected in the Scheldt Estuary, Belgium (Dumoulin & Langeraert 2020), but its establishment in Europe is currently unknown (Map 1). *Potamocorbula amurensis* has never been reported in Australia. Species range mapping from ABARES shows that the southern half of Australia, from Exmouth and Shark Bay in WA to Mackay in QLD, is potentially suitable for *P. amurensis* establishment (Map 2).

Map 1 Known global distribution of *Potamocorbula amurensis*

Data source: GBIF.org (13 May 2024) GBIF Occurrence Download: https://doi.org/10.15468/dl.8uvfps

Map 2 Maximum potential range of *Potamocorbula amurensis* in Australian waters, indicating areas of potential suitability in red, and potential unsuitability in green



Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2024

Invasion history

Potamocorbula amurensis was first collected from the inner brackish areas of San Francisco Bay in 1986 (Carlton et al. 1990). By late 1987, the clam had spread and is now an established part of the benthic community. The planktonic larvae can spend between 17 and 19 days in the water column. The pathways of spread since its introduction into San Francisco is likely by natural dispersal. Newly settled larvae and juveniles secrete byssal threads which can attach to solid particles or surfaces or to other clams. Juveniles can also be transported in accumulated sediment within fouling communities. The first record of *P. amurensis* in Europe was from the Scheldt Estuary in Belgium in 2020 (Dumoulin & Langeraert 2020).

Diseases

Specific disease data on *P. amurensis* is scarce. The propensity of *P. amurensis* to occupy degraded habitats and its efficient filter feeding behaviour means it is effective at concentrating toxins such as selenium and pesticides from the water column which have an inherent human health risk if they were to be consumed (Thompson & Parchaso 2012).

Transmissible disseminated neoplasia has been reported from other clam species *Mya arenaria* and *Venerupis corrugata* suggesting that *P. amurensis* could be susceptible (Metzger et al. 2016).

Varicorbula gibba

Varicorbula gibba is known as the European clam, the European basket shell clam, or basket shell. It is native to northwestern Europe from Norway through to the Mediterranean Sea. It has been introduced to Australia and is established in Port Phillip Bay, Victoria, and the Derwent Estuary, Tasmania (Derwent Estuary Program 2008; Wiltshire et al. 2010). It was previously detected in Port Adelaide, South Australia, but does not appear to have established there based on traditional (dredge and grab sampling) and molecular surveillance (Wiltshire and Deveney 2011, Wiltshire et al. 2022). *Varicorbula gibba* is the only introduced Corbulid to have become established in Australia (Lamprell & Healy 1998).

Varicorbula gibba is a small clam with a maximum shell length between 15–20 mm. It is an infaunal clam that is relatively immobile and sedentary, and burrows in thick, muddy sand with coarse elements. It usually inhabits the shallow sublittoral zone ranging from depths of 3 to 140 m.

The clam is not known to pose significant risks in areas it has invaded. Holmes and Miller (2006) suggested that *V. gibba* is an inferior competitor and only becomes dominant when environmental conditions become hypoxic. It was observed that *V. gibba* had significant impact on the size and growth of scallops in Port Phillip Bay (Talman & Keough, 2001). However, it is only likely to have impacts in hypoxic environments, such as those altered by human activity, and is unlikely to pose a risk in unaltered habitats under normal oxygen level conditions (Holmes & Miller 2006).

Varicorbula gibba is not nationally listed in Australia on either the APMPL or EEPL. It is included on marine pest surveillance lists for most jurisdictions in Australia, and is listed as a noxious species in some jurisdictions. A National Control Plan (NCP) was developed for *V. gibba* in 2008. NCPs were developed to assist with management of established marine pests in Australia which may have significant impacts but are deemed non-eradicable.

Table 7 Taxonomic classification of *Varicorbula gibba*

Classification	*Varicorbula gibba*
Phylum	Mollusca
Class	Bivalvia
Order	Myida
Family	Corbulidae
Genus	*Varicorbula*

Diagnostic features for identification

Field identification

The shell of *V. gibba* is small and thick and grows between 15–20 mm in length. The shell is whitish in colour with distinct variable brownish or reddish colour pattern, and is deep purple or white internally.

It is characterised by a shell that is markedly asymmetrical, with the right valve being much larger and overhanging than the left valve (Photo 6). The right valve umbo extends beyond the margin of the left valve. Sculpture on right valve is well developed and flat with moderately wide concentric ridges, while the left valve has finer, closely set raised ridges crossed by several raised radial ribs.

Both valves have well-defined post-umbonal carina. Periostracum on right valve is thin and light brown.

Photo 6 An image of two *Varicorbula gibba* specimens showing two different sizes. Note how the smaller valve fits into the larger valve for the specimen on the left.

Source*:* Evan Rees, Northern Australia Quarantine Strategy (NAQS)

Similar native species

Varicorbula gibba is unlikely to be distinguished from native Australian bivalves in the field. It is morphologically very similar to native Australian Corbulidae (*Corbula hydropica* and *C. smithiana,* and *Lentidium origolacus*), Mactridae (*Spisula trigonella* and *Mactra pura*), and Mesodesmatidae (*Paphies* spp.).

Laboratory and molecular identification

A molecular phylogenetic study by Hallan et al. (2013) included *V. gibba* using 18S rRNA and 28S rRNA genes. A qPCR assay targeting the 28S gene region was developed for this species and applied to surveillance in 2015–2016 (Deveney et al. 2017; Wiltshire et al. 2017), but returned detections in areas without known occurrence of the species, including in Darwin, where water temperature is likely to exceed the species tolerance and hence occurrence is unlikely (Deveney et al. 2017). Subsequent assessment demonstrated the 28S assay for *V. gibba* was not specific (Wiltshire et al. 2023), and a new assay for this species, targeting the COI gene region, has been developed and operationally validated (Wiltshire et al. in press). The COI assay shows high sensitivity and specificity for Australian application (Wiltshire et al. in press).

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Varicorbula gibba is a shallow burrowing infaunal clam that inhabits thick, muddy sand. It can attach to gravel and stones by a single byssal thread and is highly tolerant of low oxygen levels and survives well in polluted environments. It usually inhabits the shallow sublittoral zone ranging from depths of 3 to 140 m.

Temperature tolerances of adult *V. gibba* range between –1 to 27°C (Richmond et al. 2010). Adult growth has been observed at ambient water temperatures around the world between 8–26°C (Talman 2000). Planktonic larvae have an optimum temperature of 15–16°C (Jensen 1990).

Adult *V. gibba* can survive salinity at 0 ppt for up to two days without mortality, and up to 10 days with increasing mortality (Holmes & Miller 2006). The following salinities have been recorded for survival of this species around the world: 26–39 ppt in ambient Port Phillip Bay (Talman 2000), 28–34 ppt in ambient Limfjord, Denmark (Jensen 1990), 27–32 ppt in ambient Denmark (Jensen 1988). Planktonic larvae can survive in up to 33.5 ppt (Jensen 1990).

Varicorbula gibba is a ciliary suspension feeder that feeds on particulate organic matter, bacteria, and bottom living diatoms. Clams use the inhalant siphon to draw water containing food sources, and into the mantle cavity of the animal (Yonge 1946). *Varicorbula gibba* can compete with native bivalves for food and space. It is thought that *V. gibba* may compete with the scallop *Pecten fumatus* in deeper parts of Port Phillip Bay where *V. gibba* is abundant (Talman 1998).

Predators of *V. gibba* include gastropods, crustaceans, fish, and echinoderms. The clams are also a food source for common Eider ducks (*Somateria mollissima*) overseas.

Reproduction and growth

Varicorbula gibba has separate sexes and are broadcast spawners. In the northern hemisphere, reproduction and settlement take place in summer and autumn, although larvae have been found in winter. Growth rate of clams is about 4–7 mm per year depending on location. The lifespan of *V. gibba* is about 1–2 years, but clams can reach 5 years of age (Jensen 1990). Recruitment is possibly influenced by adult-larval dynamics, with higher numbers of adults resulting in lower numbers of recruits (Rueda et al. 2001).

Pathways and vectors

Varicorbula gibba is thought to have been transported via shipping activities. As an infaunal clam, it is unlikely to be spread via biofouling, and shipping ballast water or ballast tank sediments are the likely source of introduction into Australia. It is believed that *V. gibba* was transported to Tasmania from Victoria via domestic shipping as a secondary pathway (Derwent Estuary Program 2008). It is not known how it was introduced into South Australia.

Potential impacts

There are no reports of adverse environmental impacts in the native range of *V. gibba* (Richard Willan [MAGNT], pers. comm., April 2023). It is suggested to be an inferior competitor, especially in habitats with normal oxygen levels, and only becomes dominant when environmental conditions become hypoxic (Holmes & Miller 2006). In Australia, there was concern that densities of *V. gibba* in certain localities could “alter the ecology” of native benthic communities as it is a suspension feeder that occupies the same depth stratum as many endemic benthic species (Talman & Keough 2001). It is believed that *V. gibba* had a significant impact on the size and growth of scallops in Port Phillip Bay (Talman & Keough 2001).

In 2008, a National Control Plan (NCP) was developed for *V. gibba* in Australia. The NCP states that economic and environmental impacts of this species is likely to be ‘low.’ Overseas, there is strong evidence that *V. gibba* establishes in high densities in response to habitat degradation and hypoxia

but will not dominate in undisturbed environments. There has been little evidence of this occurring in Australian populations, however “boom and bust” cycles have been observed (Edgar, Davey & Shepherd 2009).

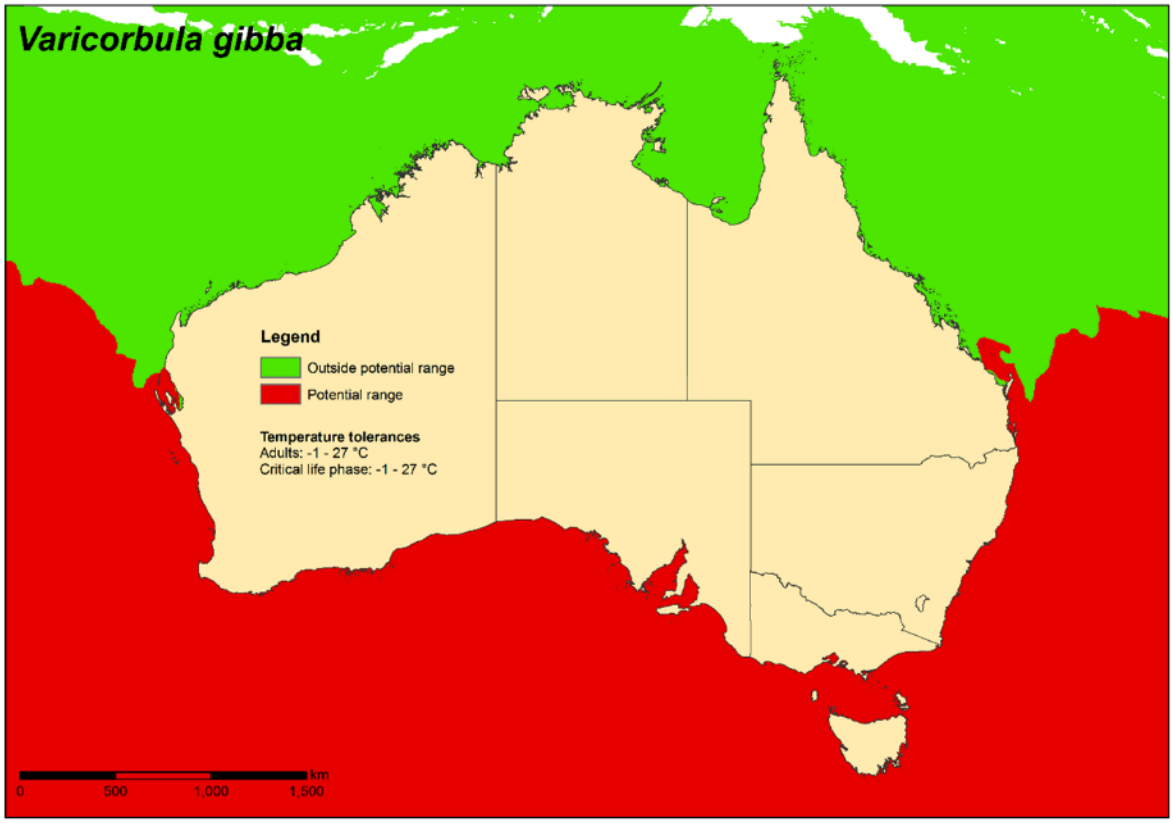
Global and Australian distribution

Varicorbula gibba is native between Norway and the Mediterranean Sea. Specifically, it has been recorded along the Atlantic Coast from the Norwegian Sea to Northern Island and the Iberian Peninsula, and is also found in the Mediterranean, Adriatic, and Baltic Seas (Mirjana 2006). The species is only known to be introduced to Australia outside of its native European range. In Australia, it was first found in Victoria, then Tasmania, then South Australia (Map 3). It is the first introduced corbulid to establish in Australia. Species range mapping from ABARES shows that the southern half of Australia, from Shark Bay in WA to Bundaberg in QLD, is potentially suitable for *V. gibba* establishment (Map 4).

Map 3 Known global distribution of *Varicorbula gibba*

Data source: GBIF.org (17 May 2024) GBIF Occurrence Download: https://doi.org/10.15468/dl.gkb79k

Map 4 Maximum potential range of *Varicorbula gibba* in Australian waters, indicating areas of potential suitability in red, and potential unsuitability in green



Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2021

Invasion history

Varicorbula gibba was introduced to Australia where it was first recorded in Port Phillip Bay, Victoria (Parry et al. 1996). After its introduction to Port Phillip Bay, *V. gibba* was recorded in the Derwent Estuary in Tasmania in 1996 (Derwent Estuary Program 2008). It was believed to be transported to Tasmania from Victoria via domestic shipping. Shells of the species, but no live individuals, were found in Port Adelaide, South Australia, in 2008 (Wiltshire et al. 2010). Follow-up surveillance using traditional and molecular methods has not detected them since in Port Adelaide (Wiltshire and Deveney 2011, Wiltshire et al. 2022) suggesting the population is no longer present. Detections were recorded at SA ports and in Darwin, NT, with the 28S assay but were considered uncertain at the time (Wiltshire and Deveney 2011, Wiltshire et al. 2022) and that assay was later shown to be non-specific (Wiltshire et al. 2023). Testing of SA and NT samples using the re-designed COI qPCR assay has not detected the species in these locations (Wiltshire et al. 2023 in press; SARDI data).

Diseases

Specific disease data on *V. gibba* is scarce. The ciliate *Sphenophyta dosiniae* has been found in living specimens of *V. gibba.* If a lamellibranch of the clam is infected with the ciliate, the ciliates will always occur in great numbers in the mantle cavity of their host (Fenchel 1965). No specific information concerning the effects of these ciliates on *V. gibba* was found.

Family Dreissenidae

Mytilopsis sallei

Mytilopsis sallei is known as the black-striped false mussel, a small bivalve native to the Caribbean Sea and Gulf of Mexico. It can heavily foul substrata in tropical and subtropical locations. It can recruit in very high numbers, causing heavy fouling on important marine infrastructure, such as wharves, pontoons, buoys, pumping stations, and aquaculture farms. This species can form dense populations in natural habitats taking up space and preventing other marine organisms from settling, leading to a reduction in biodiversity.

Mytilopsis sallei has been introduced throughout Asia (Singapore, Indonesia, India, and Japan) and the middle east (Israel and Egypt). It was detected in Darwin, Australia in 1999 but was eradicated with chemical biocides soon after. *Mytilopsis sallei* has not established in Australia but reinvasion remains a risk, particularly in tropical areas of Australia. It has been recently found on recreational vessels and foreign fishing vessels coming into Australia.

Mytilopsis sallei is nationally listed on both the Australian Priority Marine Pest List (APMPL) and on the Exotic Environmental Pest List (EEPL).

Table 8 Taxonomic classification of *Mytilopsis sallei*

Classification	*Mytilopsis sallei*
Phylum	Mollusca
Class	Bivalvia
Subclass	Heterodonta
Order	Veneroida
Superfamily	Dreissenoidea
Family	Dreissenidae
Genus	*Mytilopsis*

Diagnostic features for identification

Field identification

Mytilopsis sallei cannot be identified in the field due to high morphological variability within the species. Identification needs confirmation from internal characters under a microscope in the laboratory (Richard Willan [MAGNT], pers. comm., April 2023).

Mytilopsis sallei is a relatively small false mussel that grows to an average maximum length of 25 mm. The exterior of the shell is varied in colour, appearing white, cream-coloured, or blueish grey to a medium brown or black (Photo 7; McEnnulty et al. 2001). Some specimens may have fine concentric lines, with uncommon variants displaying black and white zig-zag markings on the shell (Photo 8; Marelli 1985). The shell is thin and easily crushed. The shell valves of *M. sallei* are slightly unequal in size with the left valve fitting inside the right one (Photo 8). A key diagnostic feature for *M. sallei* is the shape and position of the apophysis, which is a peg-like structure located insider the beak of the shell that is used to support the muscles used to close the shell (Photo 9). The septum immediately behind the umbo internally is another key diagnostic character for *M. sallei* (Photo 9).

Photo 7 Typical adult *Mytilopsis sallei* showing both sides of the shell (top), and shells at various size ranges (bottom)

Source: Northern Territory Government

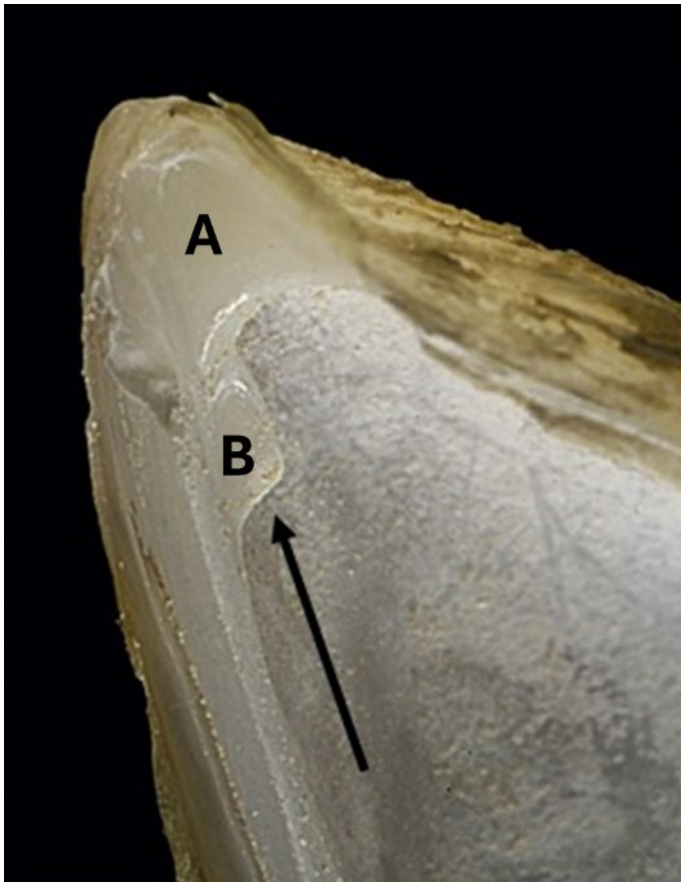
Mytilopsis sallei used to belong to the genus *Congeria*. There are currently seven extant species in the *Mytilopsis* genus, but they are poorly defined in morphological terms. The apophysis is a key diagnostic feature for the *Mytilopsis* genus. Other genera that possess apophysis are freshwater bivalves that are unlikely to occur in the marine environment (Zhulidov et al. 2021).

Photo 8 Uncommon variant of *Mytilopsis sallei* with zig-zag markings

A Black and white zig-zag markings on the shell. **B** Valves of unequal size that slightly overlap.

Source: Copyright Notice: CRIMP, CSIRO Marine Research.

Photo 9 Shell detail of *Mytilopsis sallei*



A Septum of the shell. **B** Apophysis detail of the shell (also indicated with black arrow)

Source: Marine Pest Photo album, ID confirmed by T.K. Siang, National University of Singapore

Mytilopsis leucophaeata and *M. adamsi* are both related species to *M. sallei* that are not found in Australia but have a history of invasion associated with global shipping vectors. *Mytilopsis leucophaeata* is native to the Gulf of Mexico, with populations reported from north-eastern USA and in the upper Mississippi River, along the North Sea coasts from Germany to France, and in the River Thames, England (Therriault et al. 2004). Meanwhile *M. adamsi* is thought to be native to the tropical west Pacific coast of central America and has been established in southern and eastern Asia (Marelli 2021). *Mytilopsis sallei* can be distinguished from *M. leucophaeata* by: (1) the dorsal margin of the shell in *M. sallei* is more curved than *M. leucophaeata*; and (2) the shape of the apophysis. The apophysis in *M. sallei* is large, posteriorly pointed or hook shaped (Photo 9; Marelli & Gray 1983), whereas the apophysis of *M. leucophaeata* is smaller, often rounded. *Mytilopsis sallei* can also be distinguished from other *Mytilopsis* spp. but only by expert taxonomists (see Marelli 2021). Australia has no native species belonging to the family Dreissenidae or superfamily Dreissenoidea.

Similar native species

While there are no native Dreissenidae species in Australia, *M. sallei* is morphologically similar to native Australian mytiliform species, at least superficially (notably the following genera: *Brachidontes, Mytilus,* and *Xenostrobus*). However, most native mytiliforms have features that can readily distinguish them from *M. sallei.* For example, *Brachidontes rostratus* has notable radial sculpture on its shell which is absent in *M. sallei.* In heavy fouling, *M. sallei* may superficially be confused with infestations of goose barnacles (*Lepas* spp.).

Photographs of some similar native bivalves to *M. sallei* can be found at the marine pests website.

Laboratory and molecular identification

A qPCR assay is available for the detection of *Mytilopsis sallei*. The qPCR assay is species-specific when tested on a range of other bivalve and marine species and can detect *M. sallei* in plankton samples spiked with *M. sallei* DNA (Bott et al. 2012). The assay has been applied to molecular surveillance around Australia with no detection, providing confidence in its field specificity for Australian application (Wiltshire et al. 2023). Wiltshire et al. (2023) also operationally validated this assay by testing plankton samples to which *M. sallei* tissue was added, demonstrating high diagnostic sensitivity for field application. Dias et al. (2018) have systematically undertaken taxonomic verification and vouchering of a reference *M. sallei* specimen corresponding to a species-specific short DNA sequence or 'barcode' (mitochondrial COI) of ~650 base pairs.

Refer to the guidelines for development and validation of assays for marine pests for further information see the compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Mytilopsis sallei is an epibenthic suspension feeder. It attaches to substrates using byssus threads. In its native range, *M. sallei* occupies shallow coastal lagoons. In its introduced range, it is found predominantly in sheltered intertidal and shallow subtidal habitats, usually no deeper than 2 m below low tide (Karande & Menon 1975; Udhayakumar & Karande 1989). *Mytilopsis sallei* prefers to settle on vertical surfaces and objects, but can form dense aggregations on all substrata, consisting of hundreds or thousands of individuals per m^2.

Mytilopsis sallei can tolerate a wide range of salinities and water temperature. The optimal salinity range is between 22 and 32 ppt (Ganapathi et al. 1971), but *M. sallei* can survive in freshwater (Karande & Menon 1975). *Mytilopsis sallei* is abundant in the river and monsoon drains in Singapore up to several kilometres inland from the sea (Tan & Morton 2006). Spawning in *M. sallei* is linked to a drop in salinity, with no spawning occurring above 35 ppt. Wells (2019) suggested that the osmotic shock to veliger larvae when exposed to higher salinities outside of the marinas where it was present may have reduced the risk of its potential spread in Darwin during the outbreak in 1999.

Mytilopsis sallei can survive water temperature between 5 and 40°C, however, *M. sallei* struggles at 14°C and below, with filtration rates, byssal thread production, and ability to stay attached to a surface significantly reduced (Astudillo et al. 2017). Densities of *M. sallei* in China drops between summer to winter coinciding with water temperature of 16°C (Cai et al. 2014).

Mytilopsis sallei is likely to be susceptible to desiccation because of its thin shell, however, no reliable data on this exists. Aerial exposure of another dressenid bivalve, *Dreissena polymorpha,* resulted in 100% mortality after two days of aerial exposure at 25°C (Heimowitz & Phillips 2006). The primary difference between *M. sallei* and *D. polymorpha* is that *M. sallei* naturally occurs intertidally as well as subtidally therefore could be more tolerant of desiccation than *D. polymorpha.*

Mytilopsis sallei has a high tolerance to low oxygen and is found in polluted or eutrophic areas. *Mytilopsis sallei* has been recovered from untreated organic sewage in India where dissolved oxygen was between 1.13 and 1.90 mg/L (Swami & Karande 1988).

Reproduction and growth

Mytilopsis sallei is a broadcast spawner. Sperm and eggs are released into the water column where external fertilisation takes place. *Mytilopsis sallei* is hermaphroditic. Spawning can take place year-round, although in Hong Kong, mass spawning typically coincides with a change in salinity associated with the wet season (Morton 1981). Experimentally, adults taken from normal salinity (34 ppt) and placed in lower salinity (<20 ppt), including freshwater (0.08 ppt) spawned in less than 10 hours. Spawning still occurred in salinity between 25 and 35 ppt but the time to spawning was extended (Kalyanasundaram 1975).

Females are highly fecund, releasing tens of thousands of eggs during a single spawning event. Fertilised eggs develop into pelagic larvae that settle around 8 to 10 days post fertilisation. Larval development can occur at all salinities, including freshwater (Kalyanasundaram 1975). The larvae settle by secreting byssal threads onto the settlement surface. Once settled, growth is rapid, with shell lengths reaching 20 mm after around three months (Morton 1981). *Mytilopsis sallei* are considered sexually mature at about 8 mm in size, which can be reached within one month (Morton 1989). Maximum size (40–50mm) is reached within six months of settlement and individuals live for an average of 12 to 13 months (maximum 20 months).

Pathways and vectors

Mytilopsis sallei is a fouling species and is believed to have been introduced to the eastern Pacific via vessels travelling through the Panama Canal. Several records of *M. sallei* have been made from the hulls of boats arriving in Australia (Willan et al. 2000). The most likely pathway for this mussel is hull fouling or fouling of other structures and equipment. It could be translocated by ballast water over

short distances, but because of its short larval period it is unlikely to be spread via ballast water over long distances.

Potential impacts

Mytilopsis sallei is a highly fecund species with a short larval period and rapid maturity. Therefore, this species can create heavy fouling, reaching biomass of up to 100 kg per m^2 per year in India (Rao et al. 1989). It can form layers inches thick which have to be regularly removed (Morton 1981). Biofouling can occur on all substrata, including marina infrastructure, seawater systems (pumping stations and vessel cooling systems), and aquaculture farms, with economic, environmental, and social impacts. A hypothetical incursion of *M. sallei* into Australia was estimated to result in market losses ranging from $145 million to $286 million in present value terms over a 30 year-period (Summerson et al. 2013).

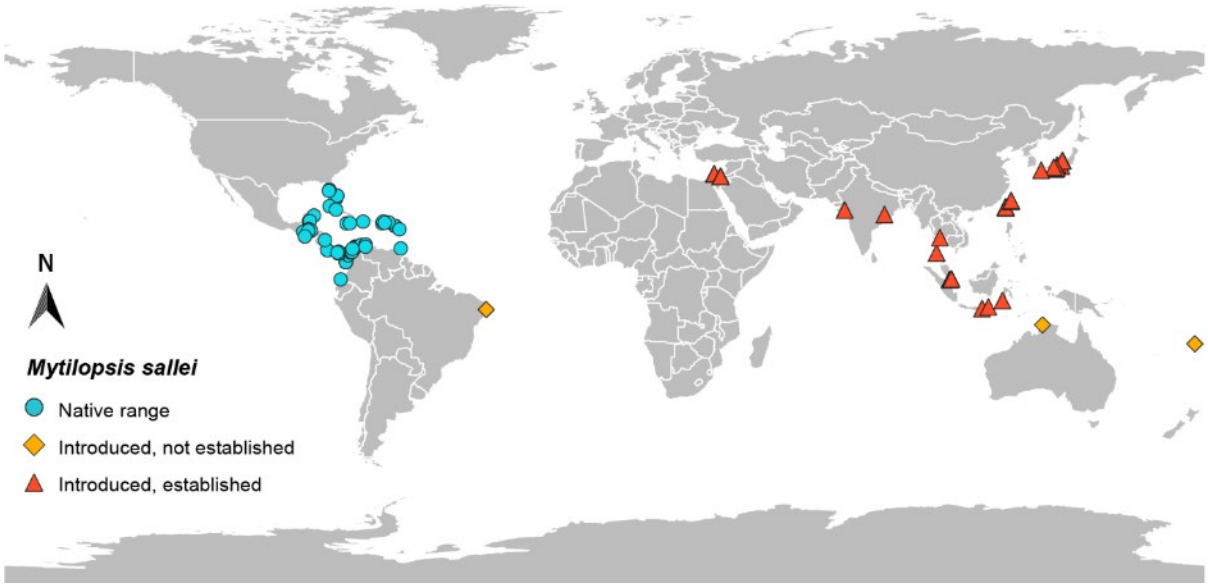
Mytilopsis sallei could disrupt Australian aquaculture production, especially other bivalve production, and fisheries activities. In south-western Taiwan, *M. sallei* causes undesirable changes in aquaculture systems and economic losses (Liao et al. 2010).

Global and Australian distribution

Mytilopsis sallei is native to the Caribbean coast of Central and South America from Yucatan to Venezuela, and part of the southern peninsula of Florida, USA. It has been introduced to several tropical and semi-tropical locations in Asia. It is established in Hong Kong, Taiwan, Japan, Indonesia, and Singapore. It has also established a population on the Mediterranean coast of Israel (Galil & Bogi 2009) (Map 5). Species range mapping from ABARES shows that the whole of Australia is potentially suitable for *M. sallei* establishment (Map 6).

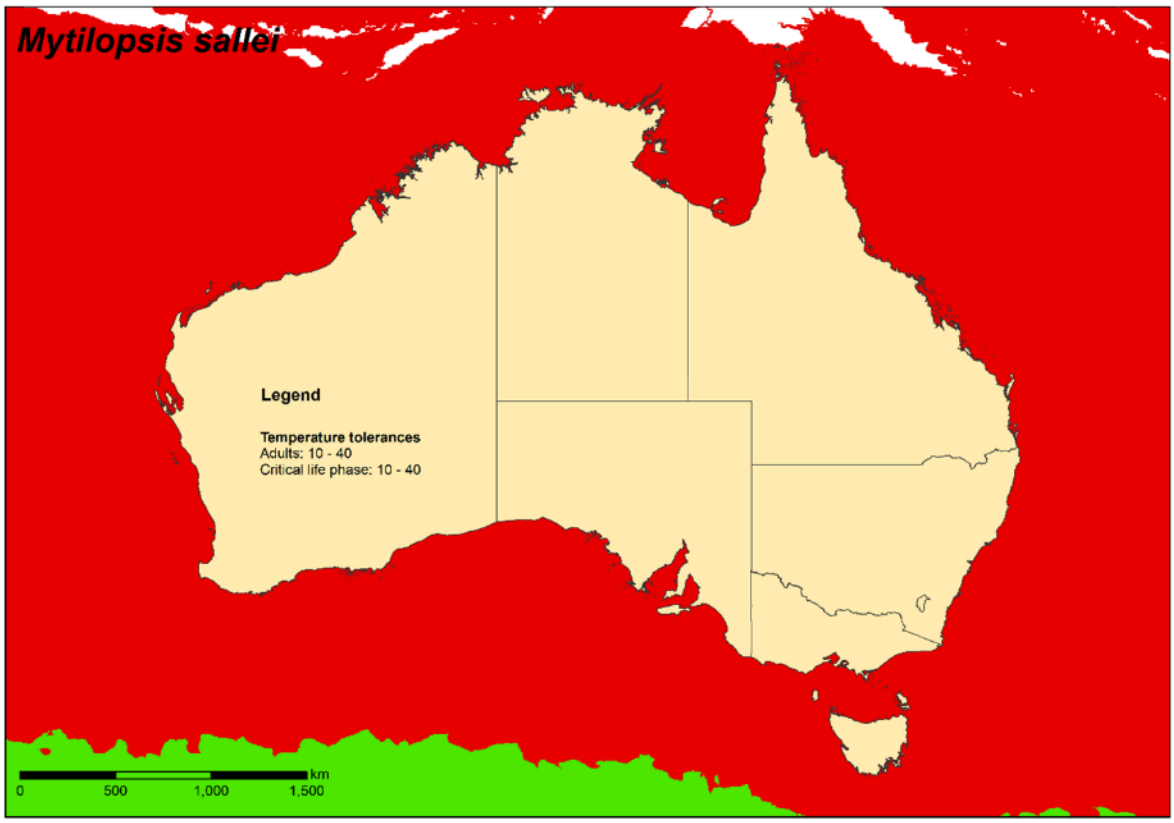
Mytilopsis sallei is not established in Australia despite several records of its occurrence as fouling on vessel hulls. A Species Range Map model suggests that all of Australia is susceptible to invasion from *M. sallei,* however the tropical areas are at highest risk. It has also been recorded in Brazil as early as 2004, however its establishment status in Brazil is not known (Queiroz et al. 2020). Reports of *M. sallei* in Fiji are disputed by taxonomists because based on the current understanding of its distribution the species is likely to be *M. adamsi*, a native to the Indo-Pacific region (Siang & Teresa 2020).

Map 5 Known global distribution of *Mytilopsis sallei*



Data source: GBIF.org (14 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.fn3pxp

Map 6 Maximum potential range of *Mytilopsis sallei* in Australian waters, indicating areas of potential suitability in red, and potential unsuitability in green



Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2021

Invasion history

Mytilopsis sallei was reported from India in the 1970s, with populations becoming established throughout ports of Asia between Japan and Hong Kong. The false mussel was introduced to China as feed for cultured fishes and shrimps in the early 1990s and shortly thereafter became the dominant species in fouling communities (Wang et al. 1999). The false mussel has also established in the tropical monsoon drains of Singapore (Tan & Morton 2006). Most recently, *M. sallei* was reported from the Mediterranean coast of Israel, having suspected to have been introduced via shipping (Galil & Bogi 2009).

An infestation of *M. sallei* in Darwin, Australia in 1999 was eradicated by chemical biocide treatments (Willan et al. 2000). The infestation in Darwin occurred in three locked marinas. The semi-contained nature of the environment where the infestation occurred assisted in the eradication of the false mussel. *Mytilopsis sallei* is not established in Australia.

Diseases

No parasites or pathogens have been recorded from *M. sallei*. The lack of pathogen data on *M. sallei* is probably a consequence of study effort rather than a lower susceptibility to infection.

A microsporidian of prawns, *Enterocytozoon hepatopenaei,* has been recorded from *M. leucophaeata* cohabitating prawn farms in Thailand (Munkongwongsiri et al. 2022), suggesting *M. sallei* may also be a susceptible pathway for this pathogen. *Enterocytozoon hepatopenaei* is an important exotic pathogen that could threaten Australia's prawn industry. The native range of *M. sallei* overlaps with major global prawn farming areas and their pathogens. An introduction of *M. sallei* to Australia could provide a pathway for other significant prawn pathogens.

Family Myidae

Mya arenaria and *Mya japonica*

Mya arenaria is known as the soft-shelled clam. It is native to eastern North America, from Canada to South Carolina. *Mya arenaria* was introduced to the Pacific coast North America, from Alaska to California. Historically, it was believed that Vikings introduced *M. arenaria* throughout Atlantic Europe as far back as 500 years ago (Essink et al. 2017).

Mya japonica is known as the Japanese soft-shelled clam and is closely related to *M. arenaria.* In 2018, taxonomic work undertaken by Zhang et al. (2018) confirmed that *M. japonica* is a separate species to *M. arenaria.* Both species are almost identical morphologically, with notable intraspecific and ontogenetic variation (Richard Willan [MAGNT], pers. comm., April 2023). Therefore, molecular diagnostics is the best way to distinguish between the two species. *Mya japonica* has been introduced to sandy beaches in the southeast of Tasmania, Australia. This is the first confirmed record of any myid species to be introduced to the southern hemisphere (Dann et al. 2020).

Both species are infaunal clams, with shell lengths between 75–100 mm, but may reach 150 mm. *Mya arenaria* has become widespread through deliberate and inadvertent human introductions, and substantial scientific literature is available for this species. In contrast, *M. japonica* is a recently recognised species with a limited introduced distribution, and therefore there are few data available. *Mya arenaria* may be used as a surrogate species where information is lacking for *M. japonica*.

Mya arenaria is nationally listed on the Exotic Environmental Pest List (EEPL), however *M. japonica* is not nationally listed in Australia.

Table 9 Taxonomic classification of *Mya* spp.

Classification	*Mya arenaria & M. japonica*
Phylum	Mollusca
Class	Bivalvia
Order	Myida
Family	Myidae
Genus	*Mya*

Diagnostic features for identification

Field identification

The genus *Mya* can possibly be identified in the field, however not to a species level, as *Mya* spp. are also morphologically similar to some native species. Identification to confirm between *M. arenaria*, *M. japonica,* or any other myid species would require molecular diagnostics. *Mya arenaria* and *M. japonica* are almost morphologically identical and molecular methods are the only method to distinguish them conclusively (Zhang et al. 2018).

Mya arenaria and *M. japonica* have a near identical appearance (Photo 10 and Photo 11, respectively). Both species have a grey or chalky-white shell that is thin and brittle, and can have a greyish/brownish periostracum. Adults grow to between 75 and 100 mm, but can reach 150 mm.

The shell shape is oval, rounded, and slightly elongate in outline with gaps at both ends for the foot and siphons of the clam.

The shell hinge has a spoon-shaped tooth (chondrophore) on the left valve which is distinctive of the genus *Mya* but non-diagnostic for differentiating *M. arenaria* and *M. japonica* (Photo 10; Zhang et al. 2018). *Mya* spp. also have a deep pallial sinus that extends deep into the shell (Photo 10). The shell has a rough sculpture marked by concentric lines (growth lines).

The siphon of *M. arenaria* and *M. japonica* may extend for as much as 200 mm to reach the surface. The siphons are tan or brown and are fused together into a single thick "neck" that is oval in cross section (Photo 12).

Although Zhang et al. (2018) identified some morphological differences between the two species, there is significant intraspecific and ontogenetic variation in *M. arenaria* and *M. japonica* which prevents accurate morphological identification (Richard Willan [MAGNT], pers. comm., April 2023). *Mya arenaria* and *M. japonica* are visually identical and molecular methods are the only method to distinguish them conclusively.

Photo 10 Adult *Mya arenaria* showing the outside and inside of the shell

A Spoon-shaped tooth on the left valve. **B** Deep pallial sinus that extends deep into the shell.
Source: Ashley Coutts/Biofouling Solutions, for the Marine Pest Photo Album

Photo 11 Adult *Mya japonica* showing the outside and inside of the shell

Source: Simon Grove, Tasmanian Museum and Art Gallery (TMAG)

Photo 12 Adult *Mya japonica* with the siphon protruding

Source: Simon Grove, Tasmanian Museum and Art Gallery (TMAG)

Similar native species

While there are no native myids in Australia, *Mya* spp. can be confused or misidentified with some native Australian clams, notably *Panopea australis*, *Lutaria rhynchaena, Venerupis* spp., and *Laternula* spp.

Photographs of some similar native bivalves to *Mya arenaria* and *M. japonica* can be found at the marine pests website.

Laboratory and molecular identification

Three published PCRs are available for the detection of *Mya arenaria*. These include two conventional PCRs, with one targeting the mitochondrial 16S rDNA gene (Ardura & Zaiko 2018) and the other targeting the mitochondrial COI gene (Hare et al. 2000). A qPCR targets the mitochondrial COI gene (Andersen et al. 2018).

A qPCR assay has been designed for *Mya japonica* using the mitochondrial COI gene (Giblot-Ducray et al. 2022). The assay was shown to be specific against DNA of other native bivalves and to be able to detect *M. japonica* in plankton samples collected from areas of occurrence in Tasmania (Giblot-Ducray et al. 2022). Further validation of this assay by Wiltshire et al. (2023) included HTS testing of samples with *M. japonica* detections and testing of archived DNA of plankton samples from around Australia to confirm assay specificity. Wiltshire et al. (2023) also tested plankton samples to which *M. japonica* tissue was added to quantify operational performance.

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Mya arenaria and *M. japonica* are infaunal clams burrowing into soft sediment, up to 50 cm deep. The shell of *Mya* spp. is thin, so they prefer finer than coarser sediment to avoid damaging the shell and for ease of mobility. Clams can move with the tide and wave action. Usually, the highest densities of *M. arenaria* occur in the high intertidal zone. The distribution of the clam in a localised beach environment is usually the result of wave hydrodynamics than other factors (Abraham & Dillon 1986). Introduced *M. japonica* in the Prosser River, Tasmania, occur in muddy intertidal areas.

Mya arenaria tolerates a wide range of salinities and temperatures and has high resistance to the presence of sulphides and low oxygen concentrations in the environment. The lowest mean salinity at which it exists in the Gulf of Bothnia is 4.5 to 5.0 ppt. Adults can tolerate salinities down to 5 ppt and up to a maximum of 35 ppt, and temperatures from –2 to 28°C, and it can survive in an oxygen-free environment for up to 8 days (Cohen 2011). For *M. japonica,* recorded temperature ranges are between 1 to 25°C (Zhang et al. 2018), however salinity and dissolved oxygen tolerances are unknown.

Mya arenaria and *M. japonica* are infaunal benthic suspension feeders. Clams feed by filtering organic material and suspended particles in the water column through their inhalant siphons.

A wide range of predators have been documented for *M. arenaria*. These include skates and rays, carnivorous bony fishes, crabs, gastropods, birds, and seals. It is likely that natural predators will be

similar for *M. japonica* in Tasmania. It should be noted that *M. arenaria* can be a valuable human food item in some European countries, and they are a commercially important species in the US.

Reproduction and growth

Mya arenaria and *M. japonica* have separate sexes and are broadcast spawners. There are no direct studies on the reproduction of *M. japonica.* However, *M. arenaria* is highly fecund with females producing an average of 100,000 to a million eggs in a single breeding season (Brousseau 1978). Generally, spawning of *M. arenaria* is temperature dependent. In its native range it spawns during the boreal spring and summer when the water temperature is usually between 10 and 20°C.

The optimal water temperature for larval development in *M. arenaria* is 20°C±3°C. Larval development time increases as the water temperature drops. Water temperature below 10°C is suboptimal for larval development. The development from fertilised egg to feeding larvae can take about 12 hours, however, it could be faster if the water temperature is warmer (Abraham & Dillon 1986).

The time from planktonic larvae to metamorphosis is approximately between 14 and 21 days. It takes around five years for *M. arenaria* to reach sexual maturity, however clams can live to 10–12 years or even longer (Abraham & Dillon 1986). For optimum growth rates, *M. arenaria* prefer clam densities below 270 clams per m^2. In its native range, 2 mm growth in juvenile clams was observed in 35 days and it took 95 days to reach 12 mm (Abraham & Dillon 1986).

Pathways and vectors

Mya spp. are benthic infaunal clams and are unlikely to be translocated by hull fouling or among other biofouling communities. *Mya arenaria* has been reported from sediments in ballast tanks (Briski et al. 2011) highlighting this as an important method of transportation. *Mya arenaria* was accidentally introduced into California via a transhipment of oysters (Zhang et al. 2018). Translocation via aquaculture is less likely to be a high-risk pathway for the introduction of *M. japonica* into Australia because of strict import requirements, but it could be an important secondary domestic pathway.

Potential impacts

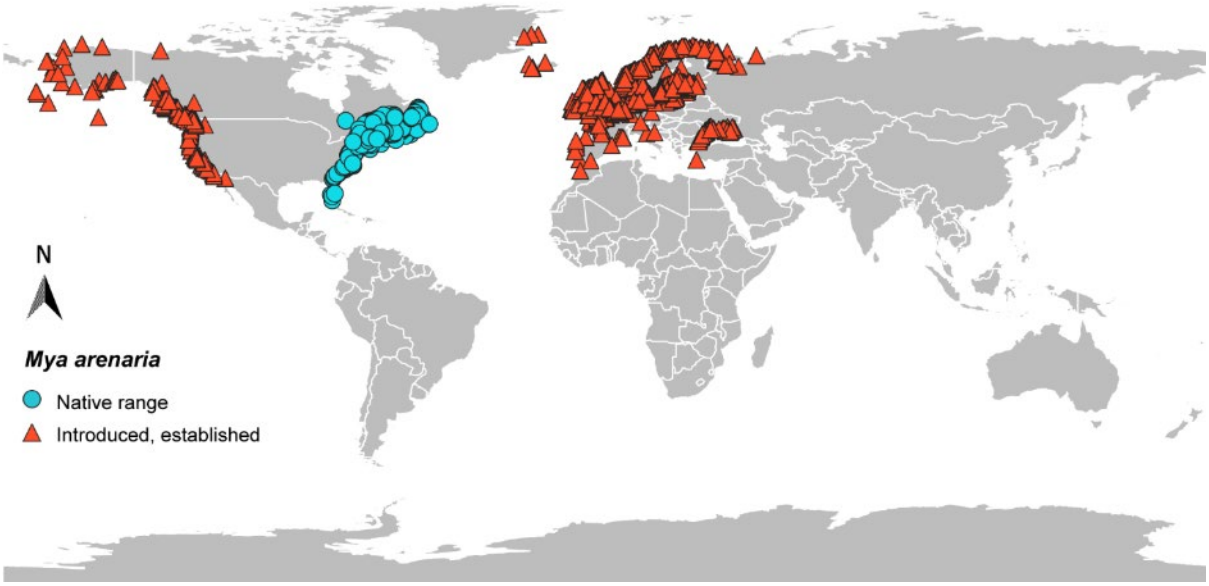
As a burrowing bivalve, it is thought that *M. arenaria* can alter surrounding benthic communities by displacing native species and outcompeting other species for food and space. However, these impacts are likely to only occur in high densities. *Mya arenaria* competitively excluded the native bivalve, *Lentidium mediterraneum,* within the Black Sea (Gollasch & Leppäkoski 1999). *Mya arenaria* may also impact bioturbation in its introduced range, altering sulphur reduction rates in the sediments surrounding its burrows (Hansen et al. 1996). In high densities, *M. arenaria* can cause social impacts when large populations of them die or wash ashore creating pungent odours (Gollasch & Leppäkoski 1999). There are currently no known impacts of *M. japonica* in its introduced range in Tasmania.

Global and Australian distribution

Mya arenaria is not recorded in Australia, however it does have the potential to be introduced into Australia. *Mya arenaria* is native to the Atlantic coastline of North America from South Carolina, USA, northwards to Canada (Map 7). *Mya arenaria* has been introduced to Pacific North America,

from Alaska to California and Atlantic Europe (including Iceland) and the Mediterranean (Crocetta & Turolla 2011). *Mya arenaria* is also present in the Black Sea in Romania (Gomoiu et al. 2002).

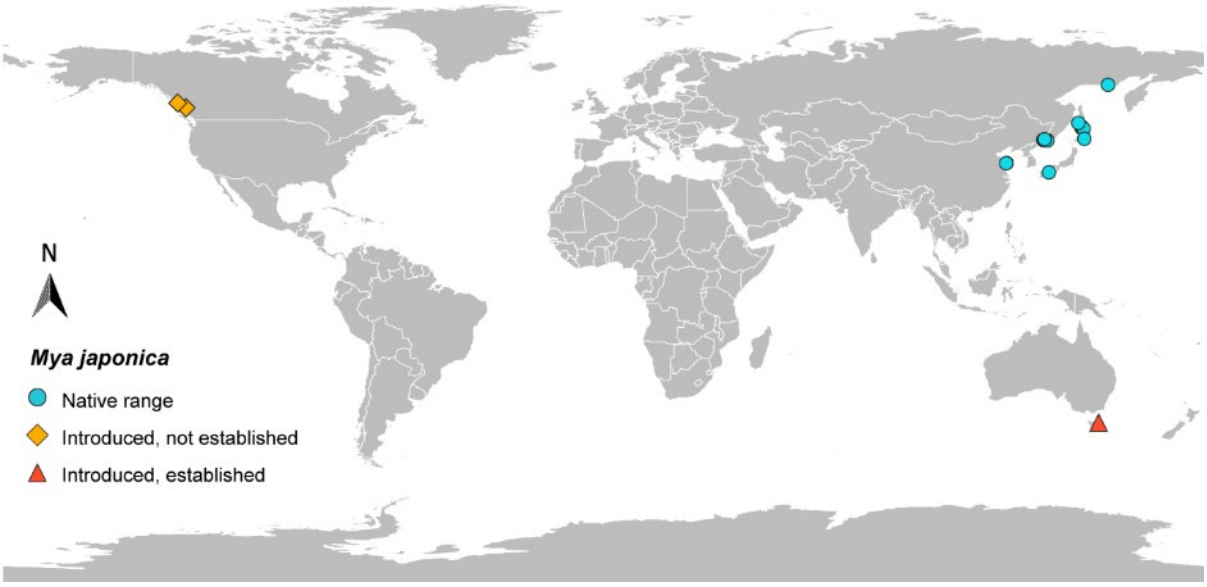
Map 7 Known global distribution of *Mya arenaria*



Data source: GBIF.org (19 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.uv4c7t

Mya japonica is native to northeast Asia, notably in Japan, China, Korea, and Russia (Zhang et al. 2018). Two isolated individuals have been identified in British Columbia, Canada, but it is not known if they have established in this location (Zhang et al. 2018). In 2018, *M. japonica* was detected in the Prosser River in Tasmania, Australia, where it has now established (Dann et al. 2020; Grove et al. 2018; Map 8). This represented the first known introduction of any myid into the southern hemisphere (Dann et al. 2020).

Map 8 Known global distribution of *Mya japonica*



Data source: GBIF.org (19 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.3hdvqb

Invasion history

Mya arenaria is native to Atlantic coast of North America. *Mya arenaria* was first reported in the Pacific coast, North America, in 1874 when it was found in San Francisco Bay, California. After its introduction it rapidly became abundant and widespread in the area. *Mya arenaria* was most likely translocated deliberately along the Pacific coast of North America up to British Columbia, Canada. *Mya arenaria* was found as far north in Alaska by at least the early 1900s, where it is now considered established. *Mya arenaria* has struggled to extend its range southward from its initial introduction point of San Francisco despite translocation attempts.

Mya arenaria is likely to have been introduced into Europe by the Vikings returning from North America (Essink et al. 2017). The clam was first reported in France in 1700 (Leppäkoski et al. 2013) and the Netherlands in 1765 (Wolff 2005). From there it has extended its range mainly northwards but also in the Mediterranean. By 1900, *M. arenaria* was found to be abundant in the Baltic Sea and established along coastlines of Sweden. *Mya arenaria* extends into the inner Baltic up to Finland and is found in Iceland. The clam's distribution also extends northward towards the poles, where it is found all along the coast of Norway up to the White Sea of northern Russia. In the southern part of its range, *M. arenaria* has a spotty distribution in the Mediterranean Sea, with established populations in the Gulf of Saronicos, Greece (Zenetos et al. 2005). *Mya arenaria* was found in the Black Sea in 1966, where it has become abundant enough to be considered a pest (Gomoiu et al. 2002).

Mya japonica was recorded by Zhang et al. (2018) from Quascilla Bay and Graham Island, British Columbia, Canada, based on publicly available sequences and shell photographs of two individuals. The authors speculate that *M. japonica* was introduced to British Columbia with the oyster *Magallana gigas* which was introduced from the early 1910s until the late 1940s (Carlton 1979). No information is available on whether the species has become established at either locality.

In 2018, *M. japonica* was detected in the Prosser River in southern Tasmania, Australia, where the species has now established (Grove et al. 2018). Dead shells of *M. japonica* were also found in November 2019 at Louisville, a few kilometres north of the Prosser River and closer to Triabunna. The species may have first colonised the Triabunna area and then later colonised the mouth of the Prosser Rover a few kilometres downstream, possibly via natural dispersal (Dann et al. 2020; Grove et al. 2018). The exact vector for introduction into Australia is not known but via ballast water, prior to the introduction of ballast water management, is most likely.

Diseases

Disseminated neoplasia is known from *M. arenaria* in its native range. Disseminated neoplasia is a lethal cancer that affects soft shell clams and is characterised by its transmissible nature (Metzger et al. 2016). *Perkinsus* spp. are known to affect *M. arenaria* in its native range. *Perkinsus marinus*, *P. andrewsi* and *P. chesapeaki* have been recorded in *M. arenaria* experiencing high mortality in the Chesapeake Bay, USA (Dungan et al. 2002). Only *P. olseni* is known in Australia where it impacts native abalone *Haliotis* spp. *Perkinsus* spp. have a wide host range, and it is likely that a *Perkinsus* spp. introduced with an invasive bivalve could become established in Australia. Transmissible diseases and pathogens of *M. japonica* are not well known.

Family Mytilidae

Arcuatula senhousia

*Arcuatula (*formerly *Musculista) senhousia* is known as the Asian bag mussel or Asian date mussel. It is a small, thin-shelled mussel with a maximum shell length of 30 mm. This mussel is native to the western Pacific Ocean, extending from the far eastern coasts of Russia southward to Singapore. It has been introduced to the USA, New Zealand, the Mediterranean Sea, and southern Australia. *Arcuatula senhousia* is established in Australia and has patchy but widespread populations in South Australia, Tasmania, Victoria, and Western Australia. It can burrow fully into the sediment or sit just on top of the sediment and can dominate benthic communities in some instances.

Arcuatula senhousia is not nationally listed in Australia on either the APMPL or EEPL. It was excluded from the APMPL because the species had been present in Australia for ~50 years in multiple jurisdictions, where no eradication attempts were undertaken, and no significant effects of this species observed (MPSC 2018).

This species is included on marine pest surveillance lists for most jurisdictions in Australia and is listed as a noxious species in some jurisdictions. It is also an invasive species of concern in the Mediterranean (Streftaris & Zenetos 2006) and in New Zealand. A National Control Plan (NCP) was developed for *A. senhousia* in 2008. NCPs were developed to assist with management of established marine pests in Australia which may have significant impacts but are deemed non-eradicable.

Table 10 Taxonomic classification of *Arcuatula senhousia*

Classification	*Arcuatula senhousia*
Phylum	Mollusca
Class	Bivalvia
Order	Mytilida
Family	Mytilidae
Genus	*Arcuatula*

Diagnostic features for identification

Field identification

Arcuatula senhousia cannot be accurately identified in the field as it looks morphologically similar to several native species (MPSC 2018). Identification needs confirmation by an experienced taxonomist or via molecular diagnostics.

Arcuatula senhousia is a relatively small, thin-shelled mussel with adults growing between 10–32 mm in length. The shell is glossy, smooth, and equivalve. The beak is near the anterior end, but not terminal. Shell colour is a dull olive-green or yellow-brown, with concentric purple-brown zigzag markings and red radial striae (Photo 13 and Photo 14). Periostracum colour is brown. Shell sculpture has 6–10 broad radial ridges anteriorly, while the rest of the shell has fine close concentric striae and growth pauses. Anterior to the ligament (below the umbo) there are 8–15 knob-like teeth.

When this mussel settles on soft surfaces, it can create “bags” from byssal threads which enclose the mussel and whole colonies, creating thick mats of mussels and byssus.

Photo 13 Adult *Arcuatula senhousia* showing the species' iridescent radiating bands

Source: Simon Grove, Tasmanian Museum and Art Gallery (TMAG)

Photo 14 Several *Arcuatula senhousia* in hand displaying variation in size and patterning

Source: Simon Grove, Tasmanian Museum and Art Gallery (TMAG)

Similar native species

Adult *A. senhousia* can be confused or misidentified with some native Australian mussels, notably *Amygdalum* spp., native *Arcuatula glaberrima, Modiolus* spp., and *Musculus* spp. There are also similarities to young individuals of *Mytilus galloprovincialis, Xenostrobus* spp., and *Brachidontes* spp.

Photographs of some similar native bivalves to *A. senhousia* can be found at the marine pests website.

Laboratory and molecular identification

A qPCR assay was for *A. senhousia* targeting the 28S rDNA gene (Bott & Giblot-Ducray 2011), but detections by this assay in Gladstone, Queensland, where the species is not recorded and was not found in concurrent traditional surveillance, suggested that the assay was non-specific (Wiltshire et al. 2019a). Further investigation by Wiltshire et al. (2023) demonstrated that the assay likely cross-reacts with DNA of other Mytilidae, probably native relatives of *A. senhousia* that have not been sequenced and hence are not represented in GenBank or other databases. A new assay for this species, targeting the COI gene region, has been developed and operationally validated (Wiltshire et al. in press). The COI assay shows improved sensitivity for *A. senhousia* detection in comparison to the 28S assay, as well as being suitably specific for Australian application (Wiltshire et al. in press).

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Arcuatula senhousia is found in intertidal and subtidal habitats up to 20–30 m depth. It can settle on both soft and hard substrata. Individuals usually settle in groups on soft substrates but can also foul hard substrates at lower densities. When settled on hard substrate, the mussel does not form a protective “bag” of byssus threads as it does on soft substrate. Like many mussels, it is a suspension feeder that consumes organic matter in the water column.

Adults have a temperature tolerance range from –5 to 32°C. In the laboratory, mortality occurs at 36°C (Guan et al. 1989). Optimal temperature for larval development is 10°C, with experiments showing that larvae did not enter pediveliger stage or settle at 15°C (Kimura & Sekiguchi 1994). Salinity tolerance in adults from North America ranges from 17–37 ppt (Zenetos 2016).

Its ability to settle in high density aggregations and form ‘byssal mats’ may facilitate competition with, or restrict growth of, other benthic species. On the contrary, the byssal mats may increase infaunal density and species richness as it can provide habitat for many species.

This species does not have many predators because of its habit of living below the substratum surface encased within a byssal bag, and for its predator avoidance behaviours (Castorani & Hovel 2016). Predators include numerous crab species, predatory snails, buccinid gastropods, rays, and migratory ducks.

Reproduction and growth

Arcuatula senhousia have separate sexes and are broadcast spawners, with males and females spawning at the same time. This species is known to have high fecundity with females producing up

to 137,000 eggs in a spawning event (Sgro et al. 2002). Reproduction of *A. senhousia* coincides with maximum water temperatures. In its native range in Japan, spawning usually occurs when water temperatures are between 22.5–25.5°C and salinity is <30 ppt (Inoue & Yamamuro 2000). In its invasive range in Italy, spawning usually occurs between 25–28°C (Sgro et al. 2002).

The larvae are planktonic and can remain in the plankton for up to 55 days. In the laboratory, larvae can reach pediveliger stage in 16 days under rearing temperatures of 25–30°C (Kimura & Sekiguchi 1994). The annual growth rate of larvae from recruitment is 15–20 mm (Creese et al. 1997), and adult size is reached in approximately nine months. The lifespan of *A. senhousia* is typically no longer than two years (Crooks 1996). Individuals at sizes >20 mm are capable of spawning (Kikuchi & Tanaka 1978).

Pathways and vectors

The main vectors for transferring *A. senhousia* are via biofouling on vessel hulls and ballast water, which were the likely vectors of introduction of this species into Australia. It is possible that the *A. senhousia* founder population was located in Port Phillip Bay in the 1970s, despite first records of the species occurring in Western Australia in the 1980s (MPSC 2018).

According to the NCP (2008) for *A. senhousia,* transfer with aquaculture equipment and seedstock is also considered a high-risk vector for this species. Oyster farming activities may entrain *A. senhousia* and should be considered as a potential secondary pathway of introduction. At localised scales, natural dispersal of larvae via ocean currents and tidal movements is also a likely secondary pathway. Dispersal by wading birds is also a possibility (MPSC 2018).

Potential impacts

Arcuatula senhousia can settle in high density aggregations and form thick byssal mates that may outcompete with or restrict growth of native benthic species and seagrass (Crooks 1998). It can also trap sediment and potentially alter sediment characteristics (Takenaka et al. 2018).

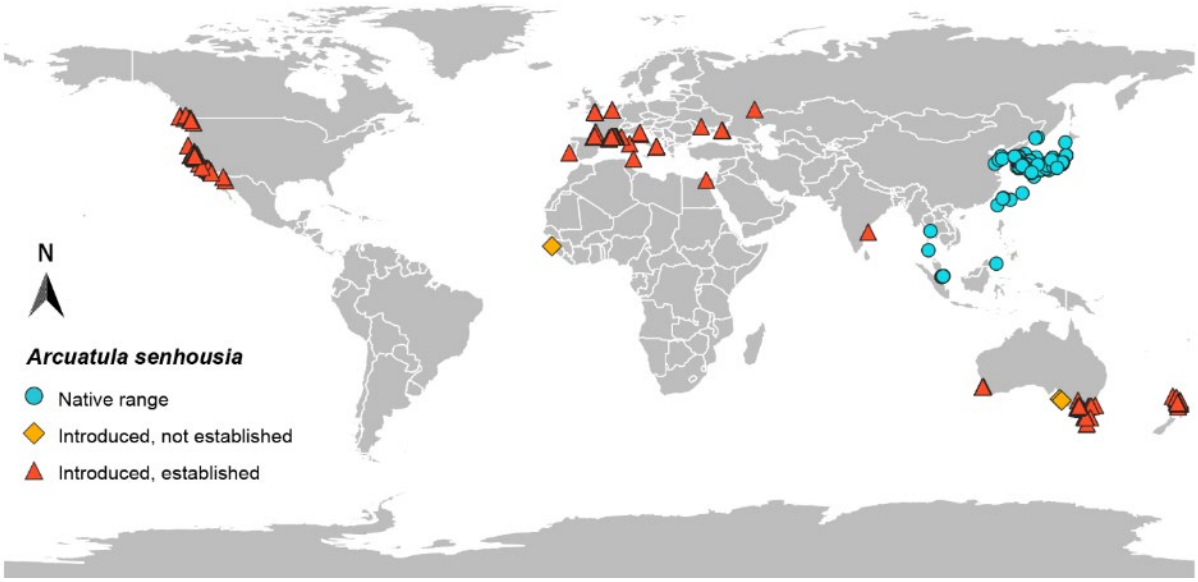
A review by Watson et al. (2021) found evidence that introduced *A. senhousia* in the UK attach to concrete tiles and empty bivalve shells. This could have implications when considering costs associated with cleaning biofouling, or transfer of mussels via aquaculture stock.

In Australia, there have been few observations of *A. senhousia* impacts in locations where it has established (MPSC 2018), and no evidence that it has formed ‘smothering anaerobic mats’.

Global and Australian distribution

Arcuatula senhousia is native to the western Pacific Ocean, extending from the far eastern coasts of Russia southward to Singapore. It is common in lagoons of Sakhalin Island, Japan, Korea, and China (Kovalev et al. 2017). It has been extensively introduced to numerous locations globally (Map 9). It has been introduced to the USA, the Mediterranean, New Zealand, and Australia. In Australia, it is found in Victoria, South Australia, Tasmania and Western Australia. There have also been detections in India and West Africa. In some locations, populations of *A. senhousia* have died out (such as in South Australia, Western Australia or Auckland) or dwindled (such as in Tasmania; MPSC 2018). Species range mapping from ABARES shows that the whole of Australia is potentially suitable for *A. senhousia* establishment (Map 10).

Map 9 Known global distribution of *Arcuatula senhousia*



Data source: GBIF.org (22 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.dd9hjd

Map 10 Maximum potential range of *Arcuatula senhousia* in Australian waters, indicating areas of potential suitability in red

Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2021

Invasion history

The first recorded invasion of *A. senhousia* was in the Pacific coast of North America in 1920s (Carlton 1979). In the 1970s, it was first recorded in the southern hemisphere in New Zealand (Willan 1985). Since 1989, it has been reported throughout European coastlines including the Mediterranean Sea, the Black Sea, in the Atlantic Ocean, in the UK, and in the Netherlands (Massé et al. 2022).

In 2016, it was recorded in the southeast coast of India in Palk Bay and is possibly established there (Behera et al. 2019). In 2018, a single specimen of *A. senhousia* was recorded in Guinea-Bissau, West Africa, but it seems unlikely that it has established at this location (Lourenço et al. 2018).

In Australia, *A. senhousia* was first recorded in the Swan Estuary, Western Australia, in the 1980s (Slack-Smith & Brearly 1987). The population in Western Australia was believed extinct after a rainfall event and toxic cyanobacterial algal bloom in 2000 (McDonald & Wells 2010), but has since re-emerged in the Swan River and Port of Fremantle (Wiltshire et al. 2020). It was also recorded in Victoria in the 1980s in Port Phillip Bay, Western Port Bay, and Portland Harbour (Parry et al. 1996). In 1995, it was reported in the Tamar River in northern Tasmania (Smith 1995) and is now present at several locations in north-east Tasmania and in the vicinity of Kettering (Grove 2018). In 1996, it was recorded in Port Adelaide in South Australia, but despite being widespread in 2001, the species was undetected in 2007–08 surveys (Rowling 2009; Wiltshire et al. 2010). The species has not been detected in Port Adelaide by subsequent traditional (Wiltshire & Deveney 2011) or molecular surveillance (Wiltshire et al. 2022), including during testing using the newly-designed COI assay (Wiltshire et al. in press).

Diseases

There are few studies on parasites, pathogens, and diseases for *A. senhousia.* Research by Miller, Inglis and Poulin (2008) found that introduced *A. senhousia* in New Zealand had lower prevalence of infection from native parasites (a copepod and a pea crab) compared to native New Zealand bivalves, *Perna canaliculus* and *Xenostrobus pulex.*

Mytella strigata

Mytella strigata is known as the Charru mussel. It is a moderately large mussel native to the tropical western Atlantic coasts of America, ranging from Panama to Argentina (Gillis et al. 2009). It is also present in tropical east Pacific coasts of America, where it is considered cryptogenic (Gillis et al. 2009). *Mytella strigata* has not been reported in Australia to-date but was previously detected and removed from a vessel arriving in northern Australia. *Mytella strigata* has been introduced into Taiwan, the Philippines, Singapore, the Gulf of Thailand, the southwest coast India, and the southeastern United States (states of Georgia and Florida).

In its introduced range, it mostly fouls man-made structures, such as docks and powerplants (Huang et al. 2021). However, it can also be found on oyster beds, shells, wood, and roots. This mussel has a wide salinity range, capable of withstanding salinity from 0 to 35 ppt. A Species Range Map model of *M. strigata* based on environmental tolerances suggests all of Australia is within the environmental range of *M. strigata* (Bloomfield et al. 2021).

Mytella strigata is nationally listed on both the Australian Priority Marine Pest List (APMPL) and on the Exotic Environmental Pest List (EEPL).

Table 11 Taxonomic classification of *Mytella strigata*

Classification	*Mytella strigata*
Phylum	Mollusca
Class	Bivalvia
Order	Mytilida
Family	Mytilidae
Genus	*Mytella*

Diagnostic features for identification

Field identification

Mytella strigata can be difficult to identify in the field and can closely resemble native Mytilid species (MPSC 2018). *Mytella strigata* has been referred to by its taxonomic synonym *M. charruana* until recently when the taxonomy of this genus was resolved (Lim et al. 2018).

Mytella strigata has an average shell length of 22–50 mm. The shell is symmetrical on both sides. Some distinguishing features of *M. strigata* include the inside of the shell which is blueish to purplish in colour (Photo 15). The sculpture on the outside of the shell valves has weak concentric striations with fine radial ribs ventrally (Photo 16). The shell is quite angular, the beak is rounded with a prominent dorsal angle, and the posterior byssal retractor muscle scar is connected to the posterior adductor muscle (Photo 17). Two small, obscure teeth are present internally at the beak (Coan & Valentisch-Scott 2012).

The external shell can have many different colours ranging from black, grey, brown, orange, and (rarely) green, occasionally patterned with zig zags, spots, or concentric bands (see Lim et al. 2018). The shell is thin and lacks exterior ribs on its shell surface.

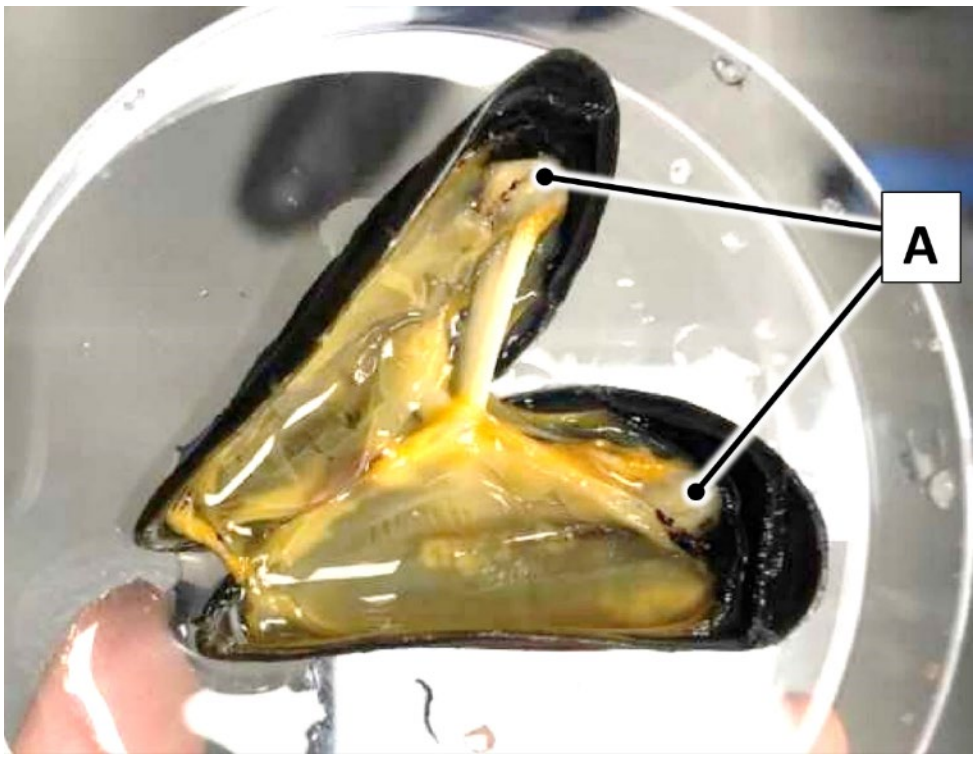
Photo 15 Adult *Mytella strigata* showing the outside and inside of the shells

Source: Ashley Coutts/Biofouling Solutions, for the Marine Pest Photo Album

Photo 16 Adult *Mytella strigata* shell detail

Source: Ashley Coutts/Biofouling Solutions, for the Marine Pest Photo Album

Photo 17 Internal view of live *Mytella strigata* specimen



A The posterior adductor muscle.
Source: Jason Bayly-Stark, Department of Agriculture, Fisheries and Forestry (DAFF)

Similar native species

Mytella strigata can look similar to native mussels (and mytiliforms) in size and shape, such as the *Mytilus edulis/galloprovincialis* complex. There has been confusion with *M. strigata* and members of the *M. edulis/gallprovincialis* complex, which have an elongate anterior pedal retractor muscle scar (MPSC 2018). Other similar species include native *Mytella* spp., *Xenostrobus* spp., *Brachidontes* spp., and *Modiolus* spp.

Mytella strigata can be differentiated from introduced species in the genus *Perna* by its blueish to purplish nacreous interior and more angled valve (Jayachandran et al. 2019; Vallejo Jr et al. 2017).

Photographs of some similar native bivalves to *M. strigata* can be found at the marine pests website.

Laboratory and molecular identification

A species-specific qPCR targeting the mitochondrial COI gene of *M. strigata* has been developed and validated under Australian conditions (Wiltshire et al. 2021). Performance of this assay was assessed in both plankton samples and in scrapes, with the latter data applicable to settlement plates (Wiltshire et al. 2021). Application of this assay to DNA from archived plankton samples collected around Australia also demonstrated field specificity of this assay (Wiltshire et al. 2021).

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Mytella strigata is common in estuarine and lagoon environments. It mainly occupies the intertidal and subtidal. It can live on a variety of surfaces, including attached to hard substrate and soft sediments. Surfaces that *M. strigata* can be attached to include rock, oysters, wood, reefs, vessels, and wharf pylons. *Mytella strigata* is unusual for a mussel in that can form dense beds either on top of, or buried into, the sediment (Carranza et al. 2009). In its introduced range in Florida, it is found on oyster beds, shells, wood, and tree roots, but is more common on man-made substrates, such as docks and intake pipes of power plants (Gilg et al. 2010).

The optimal water temperature range for *M. strigata* is between 9 and 32°C. Byssus thread production is significantly reduced at water temperatures below 13°C, likely affecting survivability of *M. strigata* at these temperatures. *Mytella strigata* can tolerate wide salinity ranges, however, the ability to tolerate salinity extremes decreases with increased or decreased temperatures. For instance, adult *M. strigata* can survive very high and low (around 2 ppt) salinities at 20°C, but their tolerance drops when the water temperature is increased or decreased. In its introduced range in Florida, populations of *M. strigata* decline or even disappear during years with cold winter water temperatures (Calazans et al. 2017).

Reproduction and growth

Mytella strigata maintains separate sexes under conditions of steady food supply, but starvation can result in the change of individuals from female to male (Stenyakina et al. 2010). *Mytella strigata* is a broadcast spawner, reaching sexual maturity at a minimum size of 12.5 mm shell length (Stenyakina et al. 2010). Spawning typically occurs during warmer water times of the year, however, trickle spawning throughout the year can occur.

Fertilised eggs develop into planktotrophic larvae and veligers where they remain in the water column for between 10 to 15 days before settling. *Mytella strigata* produce thin byssus threads to attach themselves to the substrate when ready to settle (Tay et al. 2018).

Pathways and vectors

Mytella strigata can spread via hull fouling and fouling of vessel niche areas. Vessel biofouling is the main risk pathway for this species into Australia, where mussels were previously detected and removed from a vessel's hull on arrival in northern Australia; an adult mussel in the vessel's internal seawater systems was killed by treatment. The mussel is often found attached to floating logs and other debris in its introduced range suggesting dispersal of flotsam could be an important secondary pathway. The larval period of *M. strigata* can also support its ability to be spread via ballast water. *Mytella strigata* is thought to have been introduced to Florida via ballast water in an oil tanker (Lee 1987).

Potential impacts

Mytella strigata have been observed competing with native sessile invertebrates in all countries that it has invaded (Huang et al. 2021; Lim et al. 2018). *Mytella strigata* can form high density populations, up to 49,600 m^2 and close to 100% cover, smothering other benthic organisms and aquaculture stock and clogging intake pipes of power plants (Gilg et al. 2010). *Mytella strigata* has impacted native *Perna viridis* culture in the Philippines and Singapore through high density fouling

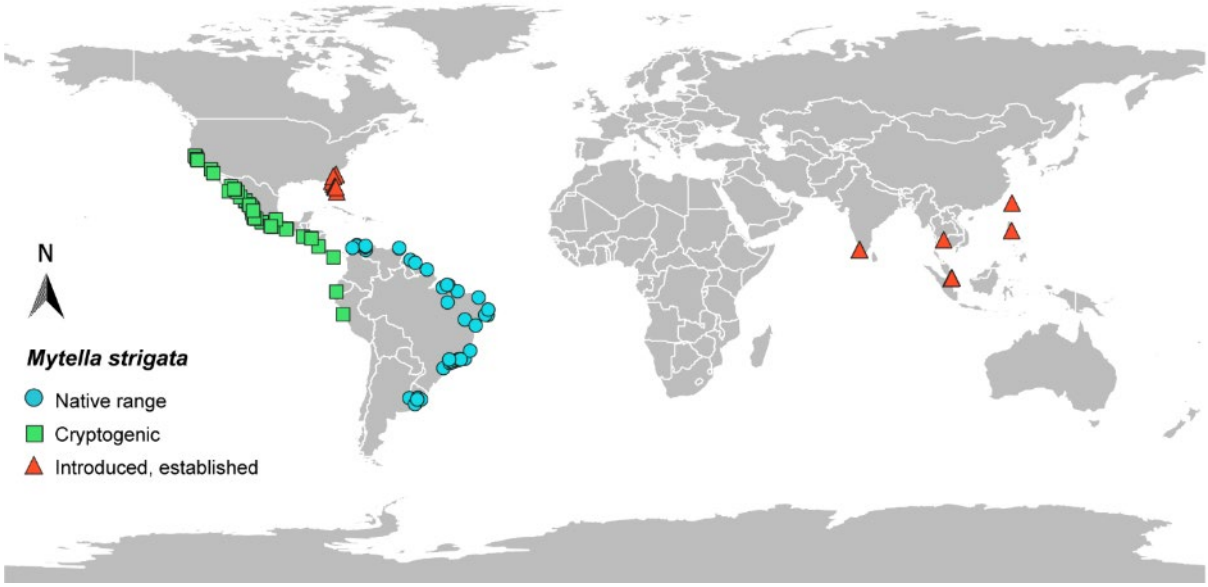
on aquaculture product (Sanpanich & Wells 2019). In Taiwan, *M. strigata* is reported to reduce growth rates and survival of cultured clams (Huang et al. 2021). It also has been observed overgrowing invasive and native mussels in the Straits of Johor and some parts of India. When fouling mussels die, they can create oxygen deprivation zones impacting benthic and mobile species.

Global and Australian distribution

Mytella strigata is native to the tropical Western Atlantic from Panama to Argentina (Gillis et al. 2009). This species is also present along the tropical east Pacific, from California to Ecuador (Gillis et al. 2009). Genetic work by Gillis et al. (2009) suggests that Pacific *M. strigata* may be a cryptic species from the Atlantic form. *Mytella strigata* has been introduced to the southeast United States, first found in Florida in 1986 and then in Georgia (Lee 1987). It has also been introduced to the Philippines and Singapore, Thailand, Taiwan, and the southwest of India (Map 11). Species range mapping from ABARES shows that the whole of Australia is potentially suitable for *M. strigata* establishment (Map 12).

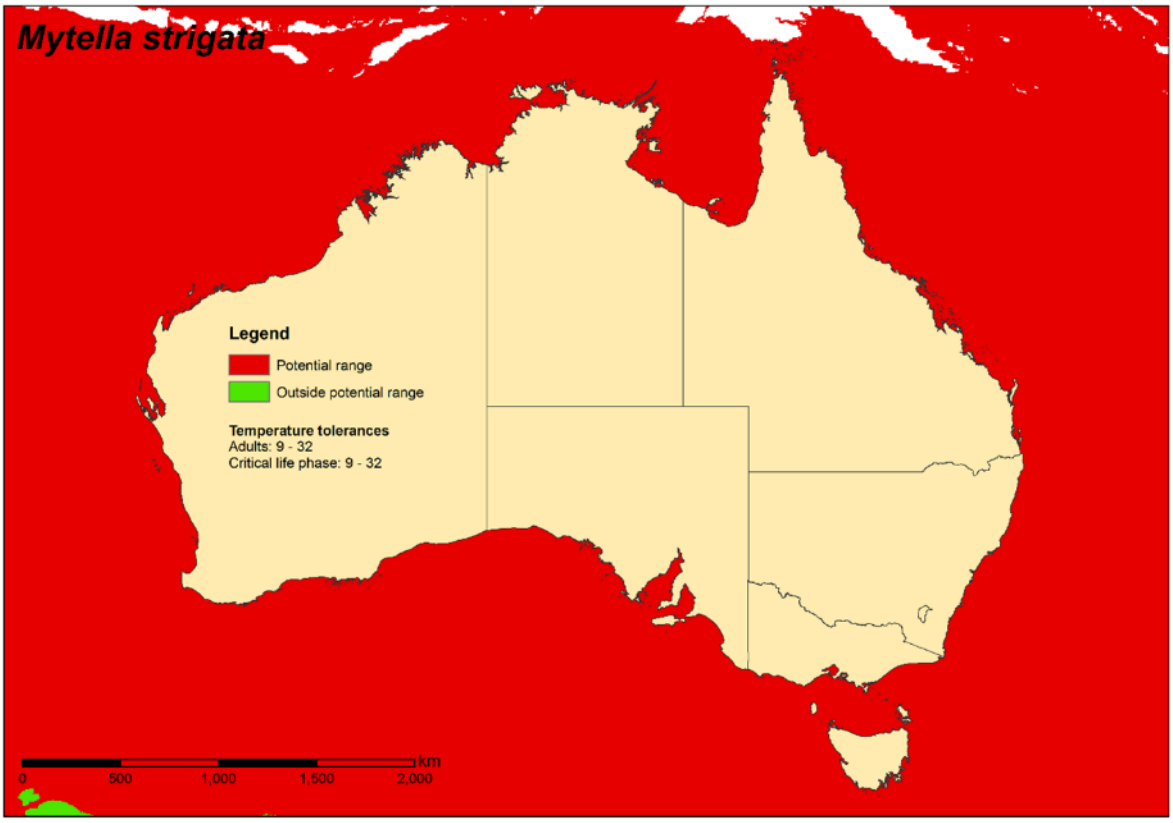
Mytella strigata has not been recorded in Australia, however, it has been detected and removed from a vessel traveling to northern Australia. The mussel has been established in several warm water lagoons and estuaries around the world. Given its preference for warmer water, it is likely a higher risk of introduction and establishment in tropical Australia than colder temperate estuaries along Australia's southern coastline.

Map 11 Known global distribution of *Mytella strigata*



Data source: GBIF.org (25 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.a8jw4u

Map 12 Maximum potential range of *Mytella strigata* in Australian waters, indicating areas of potential suitability in red



Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2021

Invasion history

Mytella strigata was first recorded in Florida, USA, in 1986 after a report of an unusual mussel clogging intake pipes of a power station (Lee 1987). The mussel was continually recorded in Florida and then later was found to have spread northwards to Georgia (Spinuzzi et al. 2013). Sporadic records have been made of *M. strigata* from South Carolina, however, the cooler winter water appears to limit its northern distribution. In 2014, *M. strigata* was reported in the Philippines (Fuertes et al. 2021). It is now found in Singapore, Taiwan, the Gulf of Thailand, and India. It is considered an important invasive pest in southeast Asia (Huang et al. 2021; Jayachandran et al. 2019; Lim et al. 2018; Sanpanich & Wells 2019; Wells et al. 2024).

Diseases

There are no species-specific disease data for *Mytella strigata*. A histopathological survey of *M. guyanensis* in Brazil revealed a range of parasites and pathogens (Ceuta & Boehs 2012). Intracellular bacteria, *Nematopsis* sp. and *Bucephalus* sp. were recorded. Mytilid mussels can be infected by several important parasites. *Marteilia refringens* and *M. pararefringens* infect *Mytilus* spp. (Kerr et al. 2018). An invasive copepod, *Mytilicola intestinalis,* has spread throughout Europe via introductions of hull-fouling mussels in the Mediterranean (Costello et al. 2021). The susceptibility of *M. strigata* to any of these parasites and pathogens is unknown but probable considering the similar life-history to other mytilid mussels.

Perna canaliculus, Perna perna, and *Perna viridis*

Perna canaliculus, *P. perna,* and *P. viridis* are large mussels and are the only members of the genus *Perna*. All three species can produce heavy fouling on fixed and floating hard substrata, including vessels, wharves, aquaculture equipment, shoreline, and reefs. Consequently, all species can damage infrastructure and alter biodiversity by outcompeting or overgrowing native species. No *Perna* spp. are established in Australia, however, detections of *Perna* spp. have previously occurred in Australia's marine environment. *Perna* spp. are occasionally detected and removed from vessels arriving in Australia.

Perna canaliculus is known as the New Zealand green-lipped mussel and is native to New Zealand. It has not successfully invaded any other countries. In Australia, it has been detected in the environment in South Australia, Tasmania, and Victoria, but populations did not establish (Furlani 1996; Glasby & Lobb 2008). It is widely cultivated in New Zealand and exported frozen around the world, and as a result, discarded *P. canaliculus* shells have been found on beaches all over Australia.

Perna perna is known as the brown mussel and is native to tropical and subtropical waters of Africa. It has been introduced to the north-western Indian Ocean, Gulf of Mexico, Caribbean Sea, and southwestern Atlantic Ocean. It has not been recorded in Australia but has been detected and removed from vessels.

Perna viridis is known as the Asian green mussel and is native to the Arabian Sea, China, India, Thailand, Malaysia, and the Philippines. It has been introduced to northern Asia, including Hong Kong and Japan, as well as the Caribbean, northern South America, and southeastern USA. In Australia, it has been detected in northern Queensland, notably Mornington Island, Amrun Port, and Escape River, however populations did not establish (Wells 2017). It is occasionally detected and removed from vessels arriving in Australia.

All three *Perna* spp. are nationally listed on the Australian Priority Marine Pest List (APMPL) and on the Exotic Environmental Pest List (EEPL). They are also included on several jurisdiction noxious species lists and surveillance lists in Australia.

Table 12 Taxonomic classification of *Perna* spp.

Classification	*Perna canaliculus, P. perna & P. viridis*
Phylum	Mollusca
Class	Bivalvia
Subclass	Pteriomorphia
Order	Mytiloida
Superfamily	Mytiloidea
Family	Mytilidae
Genus	*Perna*

Diagnostic features for identification

Field identification

Green morphs of *Perna* spp. may be identified in the field on some occasions, at least superficially (MPSC 2018). The green colour morphs are more frequently encountered in *P. viridis* and

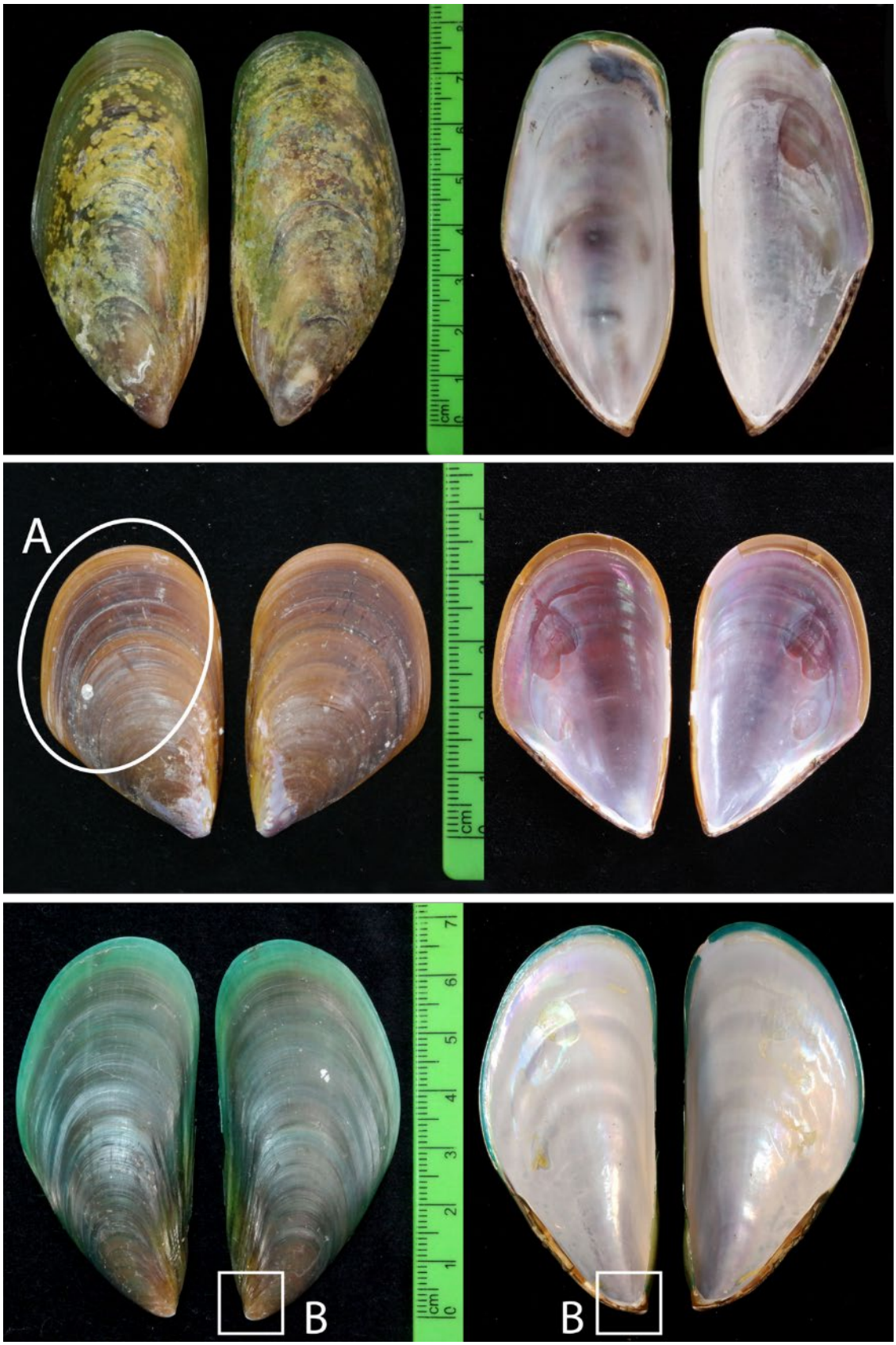
P. canaliculus, however *P. perna* also has green colour morphs (Micklem et al. 2016). Despite this, there is morphological variation within each species where the green colouration is not distinct or entirely absent in individual mussels. As a result, adult *Perna* spp. can resemble *Mytilus* spp. and juveniles can resemble *Mytilus* and *Xenostrobus* spp. Identification of *Perna* spp. needs confirmation from an experienced taxonomist or via molecular diagnostics.

Perna spp. are morphologically similar to each other (Photo 18). Green-shelled morphs of *P. perna* are almost impossible to distinguish from green *P. viridis* (Micklem et al. 2016). The classic paper to distinguish the three recent species of *Perna* morphologically is that by Siddall (1980). Rajagopal et al. (2006) provides a list of diagnostic characters between *P. viridis* and *P. perna*, meanwhile *P. canaliculus* is characterised morphologically by Furlani (1996).

The distinguishing feature of this genus from other mussel genera, particularly *Mytilus*, is the absence of the anterior adductor muscle by the beak, noting that this muscle is very small and hard to locate in many mussels (Richard Willan [MAGNT], pers. comm., April 2023). *Perna perna* is more rounded than *P. viridis* and *P. canaliculus* which are more elongate (Photo 18). *Perna viridis* has a more downwards curved beak than *P. canaliculus* and *P. perna* (Photo 18). The morphology of the posterior adductor muscles differs slightly between *Perna* spp. The most characteristic feature of *P. canaliculus* is its convex antero-ventral valve margin (Photo 19), meanwhile *P. perna* and *P. viridis* have a straight, or slightly concave, margin (Richard Willan [MAGNT], pers. comm., April 2023).

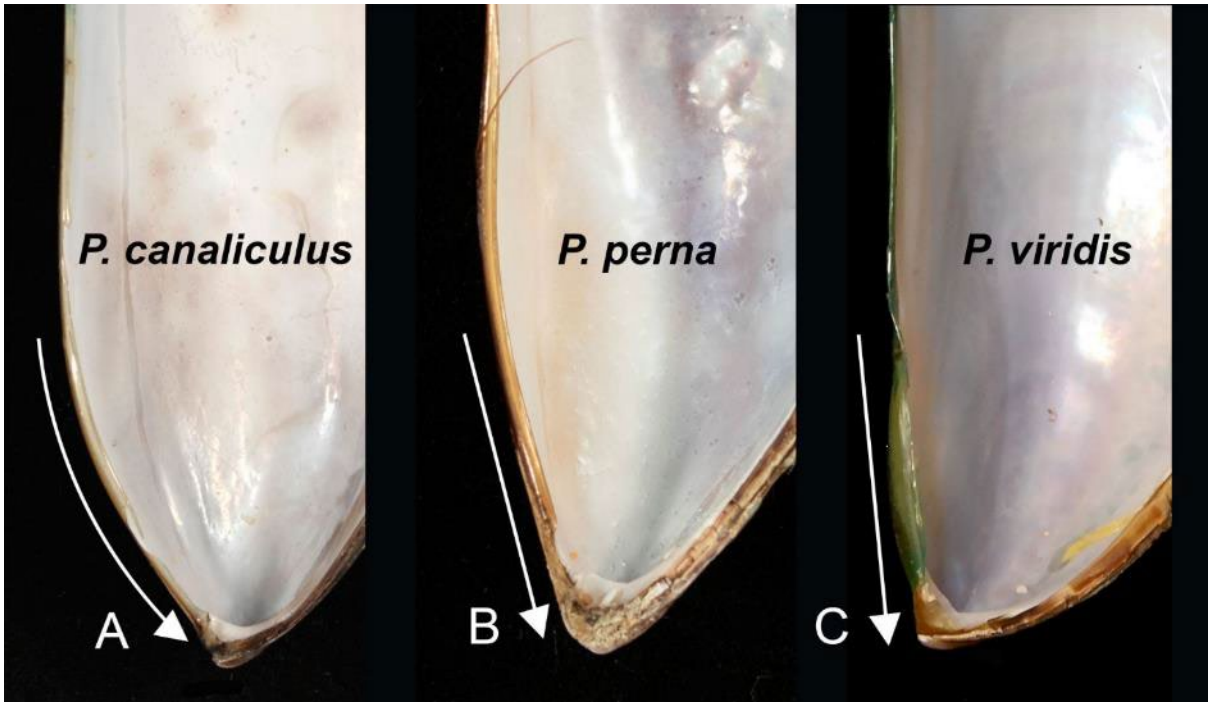
The colour is also a key feature of this genus, with all species being green-brown, although the green colouration may be absent in some individuals (Micklem et al. 2016). There can also be notable colour variation within species, including in juveniles which may display zig-zag patterning (Photo 20). Some very large adults may be covered in epibionts which can prevent the characteristic green valves from being visible. *Perna viridis* has a wavy pallial line that is not commonly observed in other the other species (see de Messano et al. 2019), however this character is not reliably seen on actual shells because they are nacreous internally (Richard Willan [MAGNT], pers. comm., April 2023).

Photo 18 Adult *Perna canaliculus* (top), *Perna perna* (middle), and *Perna viridis* (bottom)



A Rounded shell of *P. perna* compared to the elongate *P. canaliculus* and *P. viridis*. **B** Rounded beak of *P. viridis*.
Source: Ashley Coutts/Biofouling Solutions, for the Marine Pest Photo Album

Photo 19 Shape of antero-ventral valve margins in *Perna canaliculus* (left), *Perna perna* (middle), and *Perna viridis* (right)



A Convex antero-ventral valve margin of *P. canaliculus* **B** straight margin of *P. perna* **C** straight margin of *P. viridis.*
Source: Ashley Coutts/Biofouling Solutions, for the Marine Pest Photo Album, modified by René Campbell (DAFF)

Photo 20 Adult *P. viridis* (left) and juvenile *P. viridis* (right) showing variation in colour and pattern morphology

Source: Justin McDonald, WA DPIRD

Similar native species

Species of the genus *Perna* appear similar to each other, and to some other mytilids. Adult *Perna* spp. can resemble *Mytilus* spp., meanwhile juvenile *Perna* spp. can resemble *Mytilus* and *Xenostrobus* spp.

Even though live individuals of *P. canaliculus* can occasionally have a black periostracum, it can be distinguished from *Mytilus galloprovincialis* in Australia (MPSC 2018). *Perna perna* can look similar to hairy mussel *Trichomya hirsuta,* which is distinguished by its radial ridges and shell bristles. All *Perna* spp. can look similar to *Septifer bilocularis* which can have yellow-green colouration, but is distinguished by its strong radial ridges.

Photographs of some similar native bivalves to *Perna* spp. can be identified via the marine pests website below:

- *Perna canaliculus*
- *Perna perna*
- *Perna viridis*

Laboratory and molecular identification

qPCRs adapted from the literature and validated under Australia conditions exist for all *Perna* spp. Dias et al. (2013) developed primers and probe to target COI of each species, while an assay targeting the mitochondrial intergenic spacer (IGS) region for *P. canaliculus* has also been developed (Bott et al. 2011). The Bott et al. (2011) assay for *P. canaliculus*, and Dias et al. (2013) assays for *P. viridis* and *P. perna* were assessed using plankton samples with target tissue added (spiked samples) to quantify performance, and testing of archived DNA from plankton samples collected around Australia to verify field specificity (Wiltshire et al. 2023). These assays demonstrated good sensitivity and were specific when applied to Australian plankton samples (Wiltshire et al. 2023). The assay for *P. perna*, however, cross-reacted with *P. canaliculus* in spiked samples. Given no *Perna* species currently occur in Australia, the *P. perna* assay is still a useful tool, but should be applied in conjunction with testing for *P. canaliculus* or sequencing to clarify the source of any detection.

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Perna spp. have a broad temperature tolerance (Table 13). *Perna canaliculus* has a temperature tolerance between 5 and 33°C (Jeffs et al. 1999). It is more likely to survive in cooler temperate waters, as the normal temperature tolerance range of the species is between 10°C and 19°C (Ogilvie et al. 2004). Long-term lower and upper thermal limits for *P. perna* are 7.5°C and 30°C, respectively, congruent with seasonal ambient water temperature reported in other populations worldwide (Hicks & McMahon 2003). Short-term survival in *P. perna* has been observed at 42°C (Vakily 1989). *Perna viridis* has a temperature tolerance between 12 and 35°C (McFarland et al. 2015).

Perna spp. are marine bivalves that can withstand some brackish salinities. The lower salinity range of *P. perna* is 15 ppt whereas *P. canaliculus* cannot withstand salinities below 24 ppt for a long

period (Table 13). *Perna viridis* has been shown to tolerate 0 ppt salinities for up to 11 days, however, it is naturally found in salinities between 18 and 33 ppt. *Perna viridis* can also tolerate high salinity environments and is present in a hypersaline lagoon (58 ppt) in Venezuela (Segnini de Bravo et al. 1998). *Perna* spp. can be found in the intertidal and subtidal. *Perna canaliculus* has been recorded up to 50 metres deep (Jeffs et al. 1999), whereas *P. perna* and *P. viridis* are limited to around 10 metres deep.

Perna spp. have strong byssal threads allowing them to attach to substrates and withstand high wave energy environments. Laboratory experiments by Alfaro (2006) observed that *P. canaliculus* produced more byssal threads and had fewer mussel detachments under high water flow conditions compared to lower flows. *Perna* spp. attach to hard substrate, such as rocky reef, vessels, or other marine infrastructure. These mussels are rarely found on soft substrate, and they are all suspension feeders.

Perna spp. can all settle in high densities. For instance, *P. viridis* can form populations up to 35,000 individuals per m^2. Predators of *Perna* spp. include fish, seastars, crabs, and octopuses. All *Perna* spp. are an important human food source.

Table 13 Temperature and salinity ranges of adult *P. canaliculus*, *P. perna,* and *P. viridis*

Variable	*Perna canaliculus*	*Perna perna*	*Perna viridis*
Water temperature minimum	5°C	7.5°C	12°C
Water temperature maximum	33°C	42°C	35°C
Salinity minimum	25 ppt	15 ppt	0 ppt
Salinity maximum	35 ppt	55 ppt	>58 ppt

Reproduction and growth

Perna spp. have separate sexes and are broadcast spawners. Note, that *P. canaliculus* has been observed changing sex, but this is very uncommon in the wild (>0.1%). Spawning can occur year-round or at certain times of the year. *Perna canaliculus* in its native range spawn from June to December when the water temperature is between 15 and 20°C. *Perna perna* and *P. viridis* spawn when the water is warmer (Table 14). All species can grow very quickly, with *P. viridis* capable of growing up to 120 mm per year.

The typical life cycle for a *Perna* sp. includes external fertilisation of the egg which develops into veliger larvae and remains in the water column for around two weeks before settling as juveniles. However, *P. canaliculus* differs by having two settlement periods. Primary settlement involves larvae attaching to fixed or free floating macroalgae or hydroid before undergoing metamorphosis (Alfaro et al. 2004). This is thought to provide a transport mechanism for juveniles to more suitable habitat such as rocky shores. The secondary settlement involves juvenile mussels detaching from the macroalgae and settling or a hard surface where they remain for the rest of their life.

Table 14 Reproduction and growth of adult *P. canaliculus*, *P. perna,* and *P. viridis*

Variable	*Perna canaliculus*	*Perna perna*	*Perna viridis*
Water temperature range for spawning	Between 15 and 20°C	Between 20 and 30°C	Between 21 and 28°C
Age at sexual maturity	Unknown	Unknown	60 days
Size at sexual maturity	Between 27 and 50 mm	Unknown	Between 15 and 30 mm
Growth rate	60 mm per year	79 mm per year	120 mm per year

Pathways and vectors

Perna spp. are fouling species and are commonly transported on vessels or attached to other substrate. *Perna* spp. have been reported on vessels, on floating debris of unknown origin, and on natural and artificial substrates.

No known *Perna* spp. have become established in Australia to-date. The relatively long larval period of *Perna* spp. suggests they could also be spread by ballast water, particularly over short to moderate distances. *Perna* spp. are an important food item in their native ranges and intentional transportation to establish aquaculture could be an important secondary pathway in Australia.

Potential impacts

The main impacts associated with *Perna* spp. are via biofouling, as they can foul structures at high densities (Photo 21). Heavy fouling of marine infrastructure and vessels creates costs associated with control. *Perna* spp. have also been reported clogging intake pipes of land-based facilities. High density populations can also outcompete native species for space and food. *Perna perna* can both displace and facilitate other sedentary species (Hicks & Tunnell 1995).

Perna spp. can also become a nuisance species on existing aquaculture establishments through fouling of stock. In addition, as *Perna* spp. are now cultivated globally, there have been observed impacts of mussel rafts on surrounding benthic environments. For example, *P. viridis* raft-culture in Thailand resulted in changes in sediment organic matter where rafts were located compared to reference locations (Vichkovitten et al. 2017).

As a human food item, *Perna* spp. can create human health impacts when consumed, common with many other bivalves. *Perna viridis* has been recorded with high levels of accumulated toxins and heavy metals associated with poisoning in humans. *Perna* spp. like other filter feeding shellfish can bioaccumulate and retain human viruses and other pathogens that may be present in their growing waters, for example hepatitis A virus has been reported from *P. viridis* collected from Asia (Lee et al. 1999). Faecal contaminants have also been reported from *P. perna* from the Gulf of Annaba, in northeastern Algeria (Boufafa et al. 2021).

Photo 21 *Perna viridis* fouling on a rope

Source: Justin McDonald, WA DPIRD

Global and Australian distribution

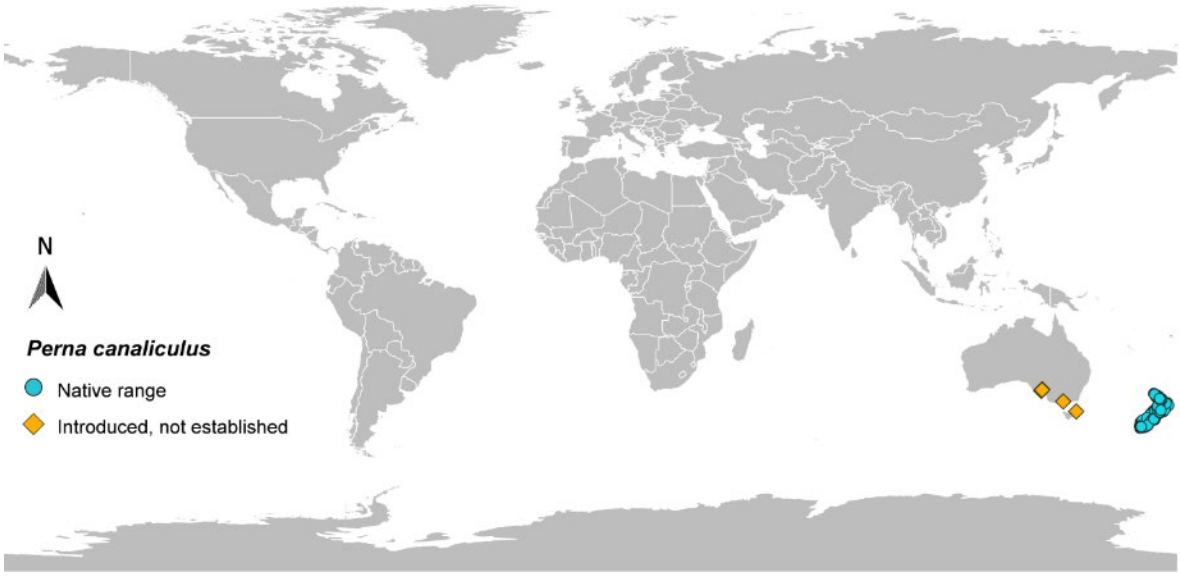
Perna canaliculus is native to New Zealand and has not established in other locations globally but has been detected in Australia on multiple occasions (Map 13). The first Australian record of *P. canaliculus* was in Bridport, Tasmania, in 1876 but the species failed to establish there. There have since been numerous interceptions over the last 20 years (Wilkens & Allen 2015). One population established in the wild in Port Adelaide, South Australia, in 1996. It is not known whether this population died out naturally or was completely removed during the eradication campaign. A single individual was also detected in Westernport, Victoria, in 2021. It is likely that *P. canaliculus* may survive in the cooler temperate waters of Australia, as the normal temperature tolerance range of the species is between 10°C and 19°C (Ogilvie et al. 2004), particularly if deliberately imported for aquaculture. However, as Glasby and Lobb (2008) noted, it would be unlikely to survive and reproduce in the Sydney estuaries (or further north) when water temperatures are warm. Species range mapping from ABARES shows that the whole of Australia is potentially suitable for *P. canaliculus* establishment (Map 14).

Perna perna has a more complex geographic range (Map 15). It is native to the western Indian Ocean (from the Bay of Bengal and the Red Sea to the tip of South Africa and as far north as Congo on the Atlantic coast of Africa). The populations of *P. perna* in Brazil and north Africa is uncertain and are classified as cryptogenic. Established populations occur in the Caribbean, Venezuela, and Israel. Genetic research by Gardner et al. (2016) identified that *P. perna* was introduced to southern India

from the Oman region. A population of *P. perna* was accidentally introduced to Tasman Bay, New Zealand, in 2007, but was subsequently eradicated (Hopkins et al. 2011). This species has not yet been detected in the marine environment in Australia but has been removed from vessels. Species range mapping from ABARES shows that the whole of Australia is potentially suitable for *P. perna* establishment, however most of the Tiwi Islands, NT, and inlets of the Prince Regent National Park region, WA, are potentially unsuitable (Map 16).

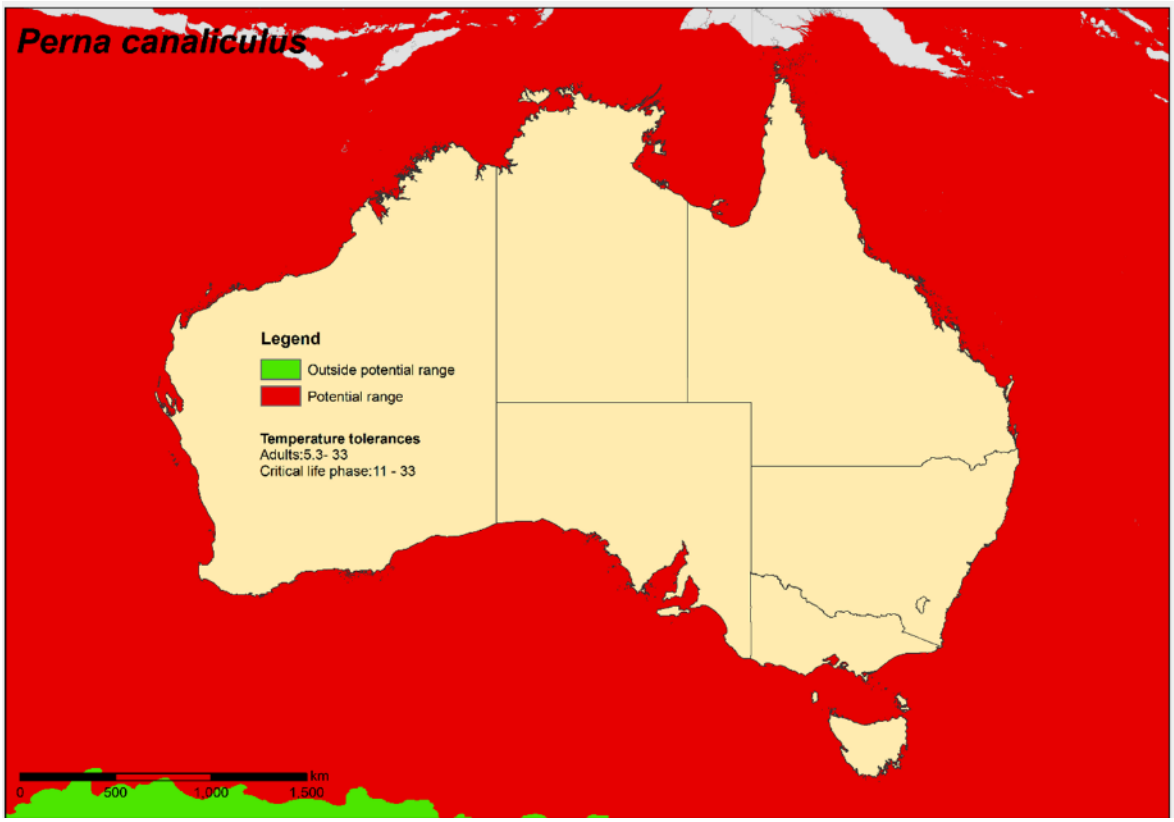
Perna viridis is native to the Indo-Pacific, from India to Thailand and south through Indonesia. It has been introduced to several parts of the world, including China, Japan, Pacific islands, the Caribbean, and southeast USA. It has been recorded in Australia several times, but no known established populations exist (Map 17). *Perna viridis* are regularly found fouling vessels arriving in Australia from Asia. Some specimens have also been recorded on driftwood, for example on Mornington Island, Queensland, and around the north coast of Australia. A small population briefly appeared in Cairns in 2001, but the infestation died out naturally (Stafford et al. 2007). In 2024, some *P. viridis* were detected in Weipa, North Queensland, and investigations on the Weipa detection are ongoing (Queensland Government 2025). Species range mapping from ABARES shows that the northern half of Australia, from Shark Bay in WA to Port Macquarie in NSW, is potentially suitable for *P. viridis* establishment (Map 18).

Map 13 Known global distribution of *Perna canaliculus*



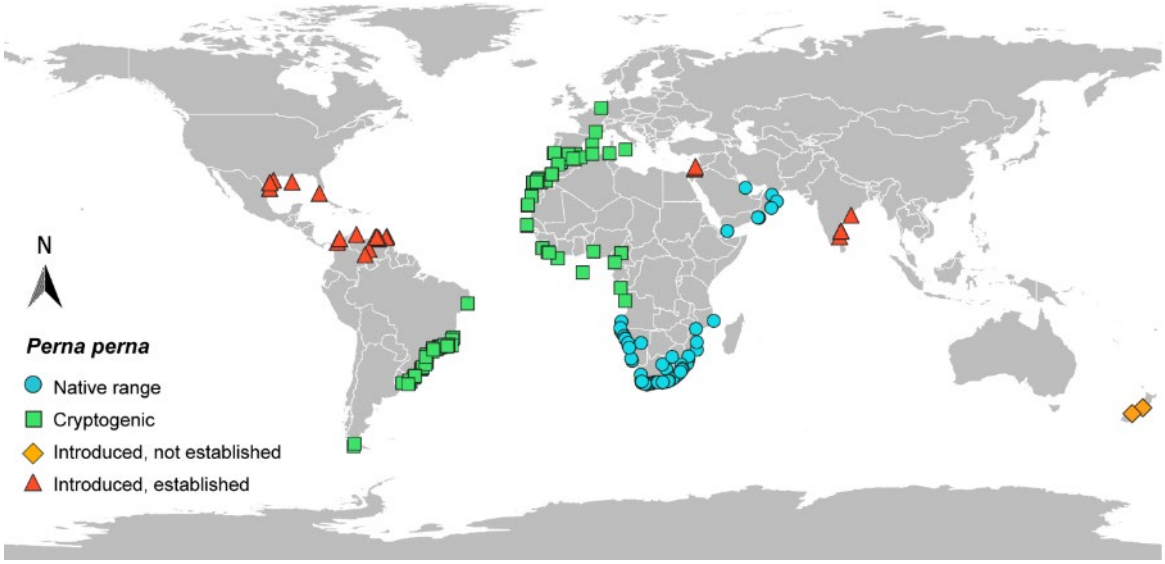
Data source: GBIF.org (26 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.aeuyf7

Map 14 Maximum potential range of *Perna canaliculus* in Australian waters, indicating areas of potential suitability in red



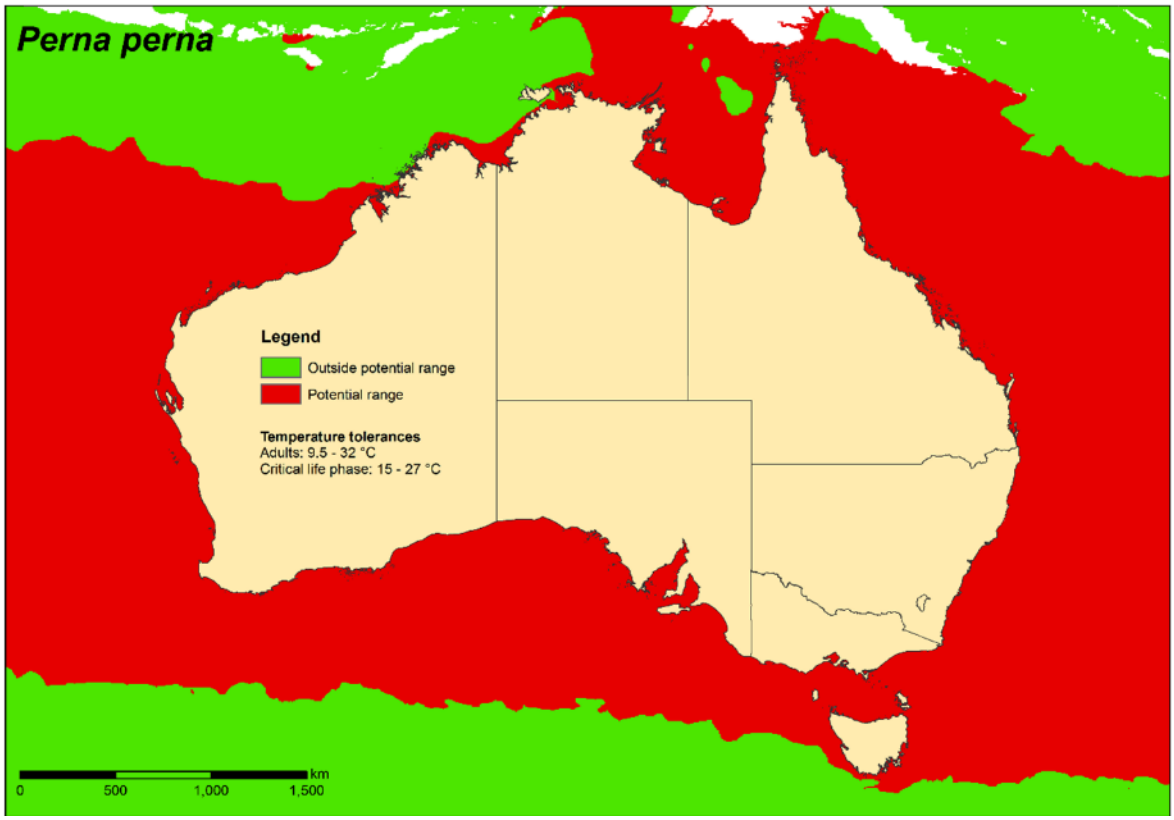
Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2021

Map 15 Known global distribution of *Perna perna*



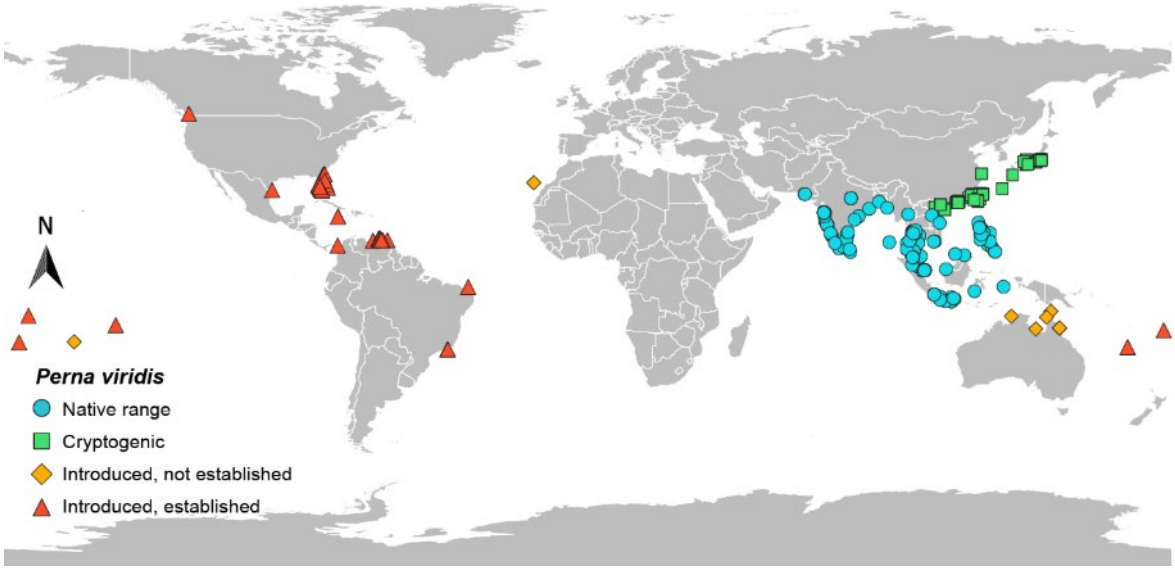
Data source: GBIF.org (26 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.uajk3x

Map 16 Maximum potential range of *Perna perna* in Australian waters, indicating areas of potential suitability in red, and potential unsuitability in green



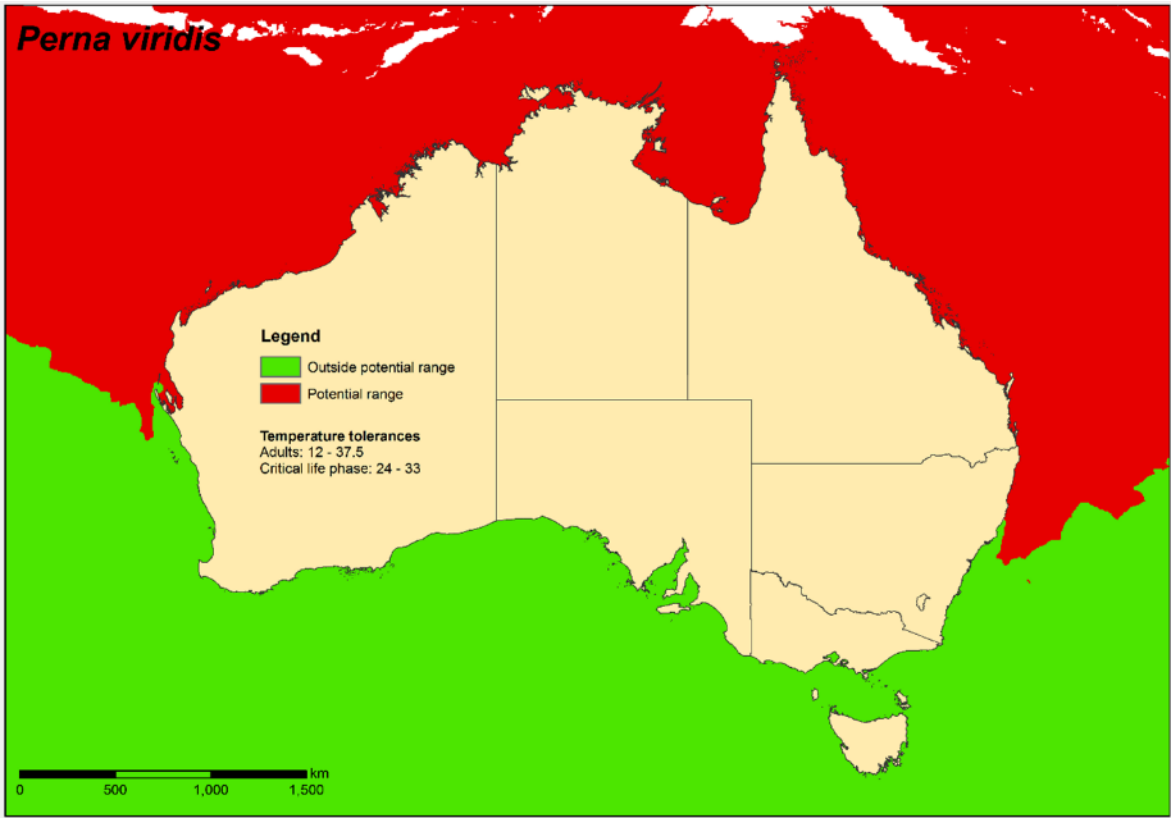
Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2021

Map 17 Known global distribution of *Perna viridis*



Data source*:* GBIF.org (26 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.y2b5sf

Map 18 Maximum potential range of *Perna viridis* in Australian waters, indicating areas of potential suitability in red, and potential unsuitability in green



Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2021

Invasion history

Perna perna and *P. viridis* have been introduced to several locations around the world. *Perna canaliculus* has failed to establish in any location outside of its native New Zealand, although it has been reported in Australia before on several occasions.

Perna viridis is native to the Indo-Pacific. Established populations were found from Tokyo Bay, Japan in 1967 that subsequently spread to other ports. The mussel subsequently spread to other locations in Asia, including China, Korea, and Hong Kong. *Perna viridis* was reported from Trinidad in the Caribbean in 1990. By 1993 the mussel had colonised several other Caribbean locations and Venezuela in South America. *Perna viridis* first appeared in the USA in the Gulf of Mexico, in Tampa Bay in 1999 when it was found fouling power plant infrastructure. The mussel spread around the Florida Peninsula and was then recorded as established in the east coast USA in 2002 when it was confirmed from the Indian River Lagoon, Florida. It was subsequently found in other locations in Florida and Georgia. Water temperature seems to limit its northern spread. *Perna viridis* has intentionally been introduced to several Pacific islands for aquaculture, with established populations present in New Caledonia, Fiji, Samoa, Tahiti, and Tonga.

Diseases

A ten-year histopathological survey of *P. canaliculus* in New Zealand found several different parasites, pathogens, or conditions of this species (Webb & Duncan 2019). These included an unknown apicomplexan parasite (APX), digestive epithelial virosis, intracellular bacteria (probably

belonging to the genus *Endozoicomonas*), *Perkinsus olseni*, *Vibrio* sp. bacteria, and flatworms *Tergestia agnostomi* and *Enterogonia orbicularis* (Webb & Duncan 2019). The record of *P. olseni* in *P. canaliculus* was first made in 2014 and is now commonly observed. *Perkinsus olseni* is present in Australia and has caused disease in Australian abalone, *Haliotis* spp. The causative agent of digestive epithelial virosis in *P. canaliculus* is unknown but suspected to be viral. The condition has also been recorded from New Zealand scallops, *Pecten novaezelandiae,* although whether this is from transmission between the two species is unknown.

Data on diseases of *P. perna* and *P. viridis* are sparse. Phototrophic endoliths, primarily cyanobacteria, infest shells of *P. perna* in South Africa. The infestation causes visible shell degradation, which can reduce reproduction and increase mortality in affected individuals (Kaehler & McQuaid 1999). Digenean trematodes belonging to the genus *Bucephalus* have been reported castrating to *P. perna* and *P. canaliculus* (da Silva et al. 2002). *Bucepahlus* spp. are common in the marine environment and through castration can impact reproduction of the host. It is probable that many of the parasites, pathogens, and conditions observed in *P. canaliculus* will be applicable to other *Perna* spp.

Castinel et al. (2019) identified parasites, *Marteilia refringens*, *M. pararefringens* and *Haplosporidium* spp., and copepods, *Mytilicola intestinalis* and *M. orientalis,* as pathogens of *Mytilus* spp. and therefore a risk to *P. canaliculus* in New Zealand.

Family Ostreidae

Magallana ariakensis

*Magallana (*formerly *Crassostrea) ariakensis* is known as the Suminoe oyster or the Chinese River oyster. It is a large, flat oyster with adults reaching shell lengths of 200–240 mm. It is native to the east coast of China but may have potentially been introduced into southern Japan. The extent of its native geographic range is not known. *Magallana ariakensis* supports regional fisheries in China, southern Japan, and possibly elsewhere in its geographic range (Hallerman et al. 2001). It was accidentally introduced to the west coast of the United States, but populations did not establish. Triploid (sterile) *M. ariakensis* were deliberately introduced to the east coast of the United States for experimental trials for potential cultivation, but these trials were ceased.

In 2023, populations of *M. ariakensis* were detected in Moreton Bay in Queensland, Australia (Queensland Government 2024), the first known detection of this species in Australia. The extent of the *M. ariakensis* population in Queensland is not currently known, and it may not be established.

Magallana ariakensis is not nationally listed in Australia on either the APMPL or the EEPL. It was excluded from the APMPL for not being readily identifiable in the field (MPSC 2018). *Magallana ariakensis* is a reportable 'biosecurity matter' in Queensland.

Table 15 Taxonomic classification of *Magallana ariakensis*

Classification	***Magallana ariakensis***
Phylum	Mollusca
Class	Bivalvia
Order	Ostreodia
Family	Ostreidae
Genus	*Magallana*

Diagnostic features for identification

Field identification

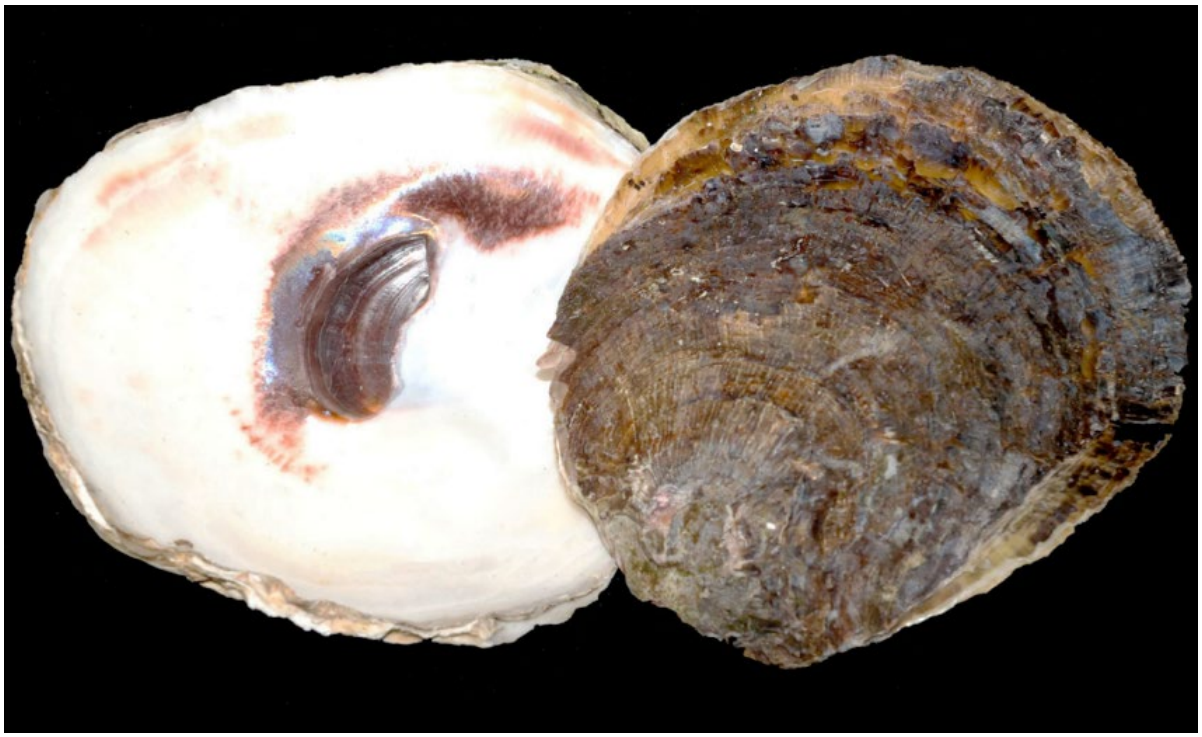
Magallana ariakensis are difficult to identify in the field at sizes below their distinctive large shell size without opening the oyster due to high external shell morphological variability, which makes differentiation between ostreid species challenging (MPSC 2018). Internal shell morphology allows identification to genus-level; however, molecular diagnostics are the recommended method for confirming species identity.

Magallana ariakensis is large and flat in appearance and can grow up to 240 mm long (Photo 22). The shell is irregular in shape with unequal valves. The muscle scar on the inner surface of the valves is large and purplish (Photo 23). As with all species of *Magallana* and *Crassostrea*, the internal shell lacks internal pits ("chomata") around the shell margin. The external shell contains flaky lamellae or 'layers', which can be grey and yellowish, or brown to purple in colour. The inner surface of the valves is smooth and greyish-white, with purple on the edges.

Photo 22 Adult *Magallana ariakensis* demonstrating its size in a human hand

Source: Queensland Government

Photo 23 Adult *Magallana ariakensis* showing the inside and outside of the valves



Source: Queensland Government

Similar native species

Magallana ariakensis are not easily distinguished from other oysters until they reach a distinctive size larger than native oyster species. This species looks very similar to the black scar oyster, *M. bilineata*, another introduced marine species in Far North Queensland. *Magallana ariakensis* can look similar to native Sydney rock oysters, *Saccostrea glomerata,* especially at smaller sizes (Photo 24).

In its native range, *M. ariakensis* appears to be part of a species complex that includes the closely-related *M. honkongensis* and *M. nippona.*

Photo 24 Juvenile *Magallana ariakensis* (left), native *Saccostrea glomerata* (middle), and mature *M. ariakensis* (right)

Source: Queensland Government

Laboratory and molecular identification

Multiplex genus and species-specific PCR markers using mitochondrial COI and nuclear 28S ribosomal RNA genes have been developed for *M. ariakensis* in China (Wang & Guo 2008). These PCRs have not been validated under Australian conditions. Sequencing has been done on *M. ariakensis* (as *Crassostrea ariakensis*) in its native range using mitochondrial COI and nuclear ITS-1 regions (Reece et al. 2008). In Queensland, Australia, partial mitochondrial 16S and COI regions have been used for sequencing *M. ariakensis.*

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Magallana ariakensis is a fouling bivalve which grows on hard objects in brackish shallow intertidal or subtidal waters, as well as muddy creeks of warm estuaries. It is found from the low tide line to 7–10 m depth but is occasionally found at the high tide mark (Zhou & Allen 2003). It can foul submerged and floating infrastructure including pylons, pontoons, and boats and can occupy disturbed habitats. In its native range in China and Japan, *M. ariakensis* is characteristic of estuarine habitats and is found in muddy intertidal zones (Zhou & Allen 2003). The extent of the *M. ariakensis* populations in Brisbane and the Moreton Bay region in Queensland are unknown, but they have bound found in similar muddy, intertidal habitats (Queensland Government 2024).

In the field, the temperature tolerance of *M. ariakensis* ranges from 2–35°C (Zhou & Allen 2003). *Magallana ariakensis* has a wide salinity tolerance ranging from 6–35 ppt (Calvo et al. 2001), however the optimum salinity range for this species is between 10–28 ppt (Zhou & Allen 2003).

Magallana ariakensis are predated on by seastars, urchins, boring snails, and crabs (Zhou & Allen 2003). It is cultivated in its native range for human consumption, and was previously proposed to be introduced into Chesapeake Bay, USA, and France for aquaculture production (Cochennec et al. 1998).

Reproduction and growth

Magallana ariakensis have separate sexes and are protandric hermaphrodites, maturing first as a male and then often becoming female later in development. They can reach sexual maturity at 2–3 months old with a shell length of 40–60 mm (Zhou & Allen 2003). Adults can reach very large sizes, up to 257 mm shell length, and can live up to 20 years (Zhou & Allen 2003). Spawning occurs at optimum water temperatures between 22–26°C, meanwhile optimum salinity for reproduction of *M. ariakensis* is 10–25 ppt in China (Zhou & Allen 2003).

Magallana ariakensis are broadcast spawners. Data on fecundity of *M. ariakensis* is scarce, but they are likely to have high fecundity like other *Magallana* spp. Eggs are fertilised externally to form ciliated trochophore larvae, and then into shelled veliger larvae. The larvae feed on phytoplankton in the water column and will develop into a pediveliger before settling on the benthos after approximately 12–18 days (Zhou & Allen 2003). In its native range, the reproductive season varies at different locations in China, with oysters able to spawn twice a year (Zhou & Allen 2003). The growth and reproductive biology of *M. ariakensis* populations in Queensland is not known.

Pathways and vectors

Like other *Magallana* spp., vessel biofouling or introductions for aquaculture (accidental or deliberate) constitutes the main risk pathways and vectors for *M. ariakensis.* This species was accidentally introduced to the US west coast with shipments of *M. gigas* from Japan (Langdon & Robinson 1996). In addition, deliberate introductions were considered to establish aquaculture production on the US east coast and France. The pathway and vectors for introduction of *M. ariakensis* into Australia is not known, but vessel biofouling is the most likely.

Potential impacts

The potential impacts and invasiveness of *M. ariakensis* in Australia is not known. However, it may foul submerged and floating infrastructure including pylons, pontoons, and boats and is able to occupy disturbed habitats. It may also present a risk as a reservoir of bivalve parasites and diseases.

Global and Australian distribution

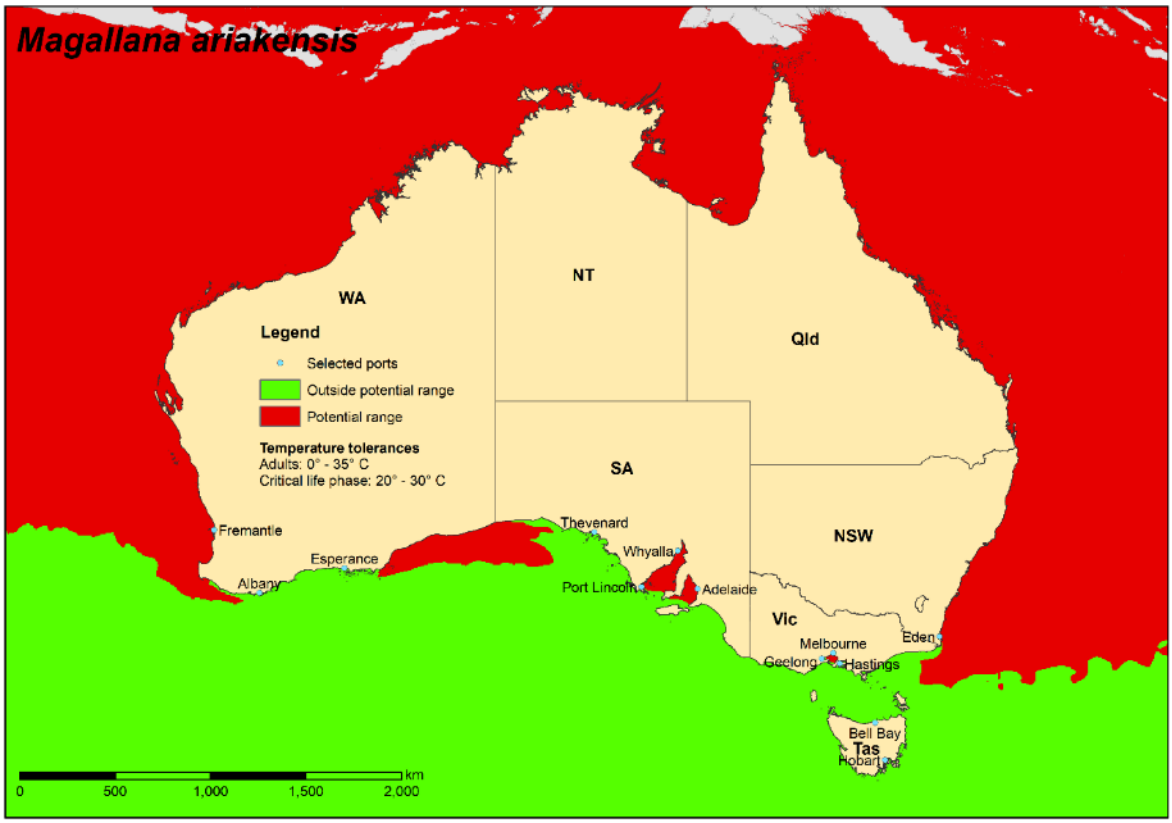
Magallana ariakensis is believed to be native to coastline of China. It was possibly introduced early to Ariake Bay in southern Japan where it was first described as *Crassostrea rivularis*. Under the name *C. rivularis,* it has been reported in India and Pakistan, and possibly Malaysia and Borneo. However, its occurrence outside of China and Japan is uncertain, and due to confused taxonomy, the extent of its native range is not known (Reece et al. 2008; Zhou & Allen 2003) (Map 19).

Magallana ariakensis was accidentally introduced to the east coast of the United States but did not establish. Triploid *M. ariakensis* were deliberately imported to the west coast of the United States for experimental trials, but these trials were ceased and most of the triploid populations have been destroyed (noting that triploids are sterile and would have been unable to establish). *Magallana ariakensis* was introduced to the Moreton Bay region in Queensland, Australia, which represents the first known detection outside of Asia. It's establishment status in Queensland is not currently known. Species range mapping from ABARES shows that nearly all of WA's coastline (except for the area roughly between Albany and Esperance), parts of SA and VIC, and the whole of NSW, QLD, and NT are potentially suitable for *M. ariakensis* establishment (Map 20).

Map 19 Known global distribution of *Magallana ariakensis*

Data source: GBIF.org (8 June 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.6s48m8

Map 20 Maximum potential range of *Magallana ariakensis* in Australian waters, indicating areas of potential suitability in red, and potential unsuitability in green



Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2024

Invasion history

Magallana ariakensis was accidentally introduced to Oregon on the United States west coast with shipments of *M. gigas* from Japan, but no wild population established (Langdon & Robinson 1996). Plantings of *M. ariakensis* were also detected in Puget Sound, Washington, but also failed to establish (Langdon & Robinson 1996).

The species garnered attention as a possible replacement or supplement for oyster populations affected by disease. Importation of *M. ariakensis* was considered in France (Goulletquer et al. 2002), and also in Virginia and North Carolina on the United States east coast. On the United States east coast, decline of the oyster *M. virginica* from disease led to interest in importing exotic, disease-resistant *M. ariakensis.* In 1998, trails were carried out with *M. ariakensis* stock obtained from Oregon hatcheries, and successful, small-scale trials led to extensive stocking of triploid (sterile) oysters into open waters of Chesapeake Bay and North Carolina. A review of *M. ariakensis* biology, including low genetic diversity of the cultured stock, susceptibility to *Bonamia* spp. infection, and risk of triploid reversion, found that the risk of cultivating *M. ariakensis* outweighed the benefits (Grabowski et al. 2007). In 2009, cultivation of triploid oysters in open waters was ceased, and introduction of diploid *M. ariakensis* was prohibited.

In 2023, *M. ariakensis* was first detected in the Moreton Bay region in Queensland, Australia (Queensland Government 2024), the first known detection outside of Asia. Specifically, it was detected at Bribie Island within Moreton Bay, and also at Boggy Creek, Pinkenba, and Kedron Brook

near Brisbane. The full extent and establishment status of the introduced population in Queensland is not yet known.

Diseases

Magallana ariakensis is susceptible to *Bonamia* spp. parasites (Audemard et al. 2008). In France, quarantined experimental populations of *M. ariakensis* experienced mortalities from a *Bonamia*-like parasite, which had not previously been seen in oysters of the genus *Magallana* (Cochennec et al. 1998). In 2003, triploid *M. ariakensis* planted in North Carolina also experienced substantial mortality from a *Bonamia* parasite, which was genetically most similar to known *Bonamia* species in Australia and New Zealand (Bishop et al. 2006).

Bonamia exitiosa has previously been detected in Australia, specifically in flat oysters (*Ostrea* spp.) in Victoria and New South Wales. *Bonamia*-like parasites that are likely to be *B. exitiosa* have also been recorded in Tasmania and Western Australia (Buss et al. 2020; Carnegie et al. 2014). *Bonamia exitiosa* is mainly a parasite of *Ostrea* flat oysters, however microcells have occasionally been detected in Pacific oysters (*M. gigas*) and Sydney rock oysters (*Saccostrea glomerata*) (DAFF 2020b).

Bonamia ostreae is exotic to Australia, but microcells have been detected in cupped oyster species such as *M. ariakensis.* Therefore, these oysters are considered naturally susceptible to infection by *B. ostreae* and could be a risk pathway (DAFF 2020b). As the detection of *M. ariakensis* in Queensland is recent, little is known about potential *Bonamia* spp. infection in this population or the associated disease transmission risks. However, disease testing on several *M. ariakensis* specimens collected from Queensland returned negative results for exotic pathogens.

Magallana bilineata

*Magallana (*formerly *Crassostrea) bilineata* is known as the black-scar oyster. It is a large tropical oyster that can grow to ~210 mm shell length and is native to the Indo-Pacific Ocean, including Pakistan, India, Sri Lanka, China, Thailand, Malaysia, Indonesia, the Philippines, Japan, and Papua New Guinea (Willan et al. 2021). *Magallana bilineata* is an important aquaculture species in the Philippines and India, and was deliberately introduced to Fiji in the 1970s for aquaculture (Kinch et al. 2019). In 2019 it was detected in Far North Queensland, Australia, where it has been found in disturbed habitats (i.e. marinas and harbours) of Cairns, Mission Beach, Mourilyan Harbour, Elim Beach, and Port Douglas, and also on rocks near unmodified anchorages near Cooktown (Willan et al. 2021).

Magallana bilineata is not nationally listed in Australia on either the APMPL or the EEPL. The potential risks of *M. bilineata* on Australian ecosystems is unknown, but it is a reportable 'biosecurity matter' in Queensland.

Table 16 Taxonomic classification of *Magallana bilineata*

Classification	***Magallana bilineata***
Phylum	Mollusca
Class	Bivalvia
Order	Ostreodia
Family	Ostreidae
Genus	*Magallana*

Diagnostic features for identification

Field identification

Magallana bilineata cannot be identified in the field due to high morphological variability, which makes differentiation between ostreid species challenging (Dridi et al. 2008). Therefore, molecular diagnostics are the recommended method for confirming species identity.

The shell of *M. bilineata* is variable in shape, usually elongate or circular, and sometimes possesses lateral 'lobes'. This species can grow up to 180-212 mm long. Characteristic of *M. bilineata* is a very dark purple/black adductor muscle scar (Photo 25), which is the distinguishing feature of this species (Willan et al. 2021). Some other species may, however, variably possess this trait.

The external shell is usually pale yellow or purple with thin, flaky lamellae or 'layers' (Photo 26). The lower left valve is often deeply cupped, while the right valve is nearly flat (Willan et al. 2021). As with all species of *Magallana* and *Crassostrea*, the internal shell lacks internal pits ("chomata") around the shell margin. The hinge and ligamental area are straight and short, with the ligament being deeply grooved between equal-sized segments.

Photo 25 Adult *Magallana bilineata* from Mourilyan Harbour (left) and from Cooktown (right). Note the prominent black adductor scar on the valves

Source: Evan Rees, Northern Australia Quarantine Strategy (NAQS)

Photo 26 Adult *Magallana bilineata* on rocks with valves closed

Source: Carmel McDougall, Griffith University

Similar native species

Magallana bilineata are not easily distinguished from other oysters until they reach a distinctive size larger than native oyster species. This species looks similar to wild *M. gigas*, however the ranges between the two species do not currently overlap, as *M. bilineata* is a tropical species whereas *M. gigas* is a temperate species (Ghaffari et al. 2019; Willan et al. 2021). *Magallana bilineata* may look similar to native flat oysters, *Ostrea angasi*, and rock oysters, *Saccostrea* spp.

Laboratory and molecular identification

There are currently no species-specific qPCR assays for *M. bilineata.* However, sequencing has been carried out on *M. bilineata* in Australia using universal mitochondrial COI and 16S primers (see Willan et al. 2021).

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Magallana bilineata is a fouling bivalve, and has been found attached to rocks, pylons, jetties, and boat hulls in disturbed estuarine areas in Australia (Willan et al. 2021). Elsewhere, *M. bilineata* lives in turbid, brackish waters such as estuaries, creeks, bays, ports, and harbours. It has also been found in estuaries subjected to extended periods of freshwater inflow. This species can be found from the midlittoral zone to a depth of 15–16 m and is reported to form shellfish reefs in some areas (Lau et al. 2020). Oysters will settle on a wide range of substrates, including tiles, rocks, shells, bamboo, tyres, and PET bottles.

Magallana bilineata is a tropical species that is known to tolerate high temperatures. Adults survive at a minimum temperature of 15°C (Lau et al. 2020), meanwhile the maximum temperature threshold is around 37–39°C (Rajagopal et al. 2003c; Rajagopal et al. 2015). This species can naturally occur at sites that experience salinities as low as 0 ppt (Piyathilaka et al. 2012) and can survive salinities up to 53 ppt (Rao 1974).

Magallana bilineata are predated on by several marine species such as boring snails, boring polychaetes, and crabs. It is an important aquaculture species in some parts of the world.

Reproduction and growth

Magallana bilineata have separate sexes and are sequential hermaphrodites, with both male-to-female and female-to-male transitions being recorded. The early sexual phase is usually male in most of the population (Rao 1956), however geographical differences in reproductive strategy (hermaphroditism) has been noted (Joseph & Madhyastha 1984). *Magallana bilineata* are sexually mature after one year and adults can live up to 4 years (Nayar et al. 1984). They grow at around 3 mm per month, reaching maturity at shell lengths of 12–14 mm in males, and 24–26 mm in females (Joseph & Madhyastha 1984). The lowest temperature that spawning has been recorded in *M. bilineata* is 24°C (Rao 1951), but salinity is also an important reproductive cue for this species.

Magallana bilineata are broadcast spawners and a large female measuring 80–90 mm shell length can release up to 15 million eggs (Xavier 2017). Eggs are fertilised externally and form trochophore

larvae, which will continue to develop over 14–17 days before settling on the benthos (Nayar et al. 1984). Spawning periods of *M. bilineata* differ across its geographic range, influenced by both temperature and salinity (Rao 1956), but spawning can occur year-round. Maturation and spawning occur between 23.5 and 32°C (Nayar et al. 1984). The optimal salinity range for spawning is between 20 and 28 ppt (Rao 1951).

Pathways and vectors

Like other *Magallana* spp., vessel biofouling or introductions for aquaculture (accidental or deliberate) constitute the main risk pathways and vectors for *M. bilineata.* Outside of its native range, *M. bilineata* was deliberately introduced into Fiji to establish aquaculture. In Far North Queensland, Australia, *M. bilineata* has been found in large numbers on boat hulls in several ports and marinas. This suggests that hull biofouling is the most likely vector for this species into and within Australia (Willan et al. 2021).

Potential impacts

The potential impacts and invasiveness of *M. bilineata* in Australia is not known, however, it can foul submerged and floating infrastructure including pylons, pontoons, and boats. It also has the ability to occupy disturbed habitats including shallow subtidal sites. Internationally, it is known to foul infrastructure including cooling water systems for power stations (Rajagopal et al. 2003c). *Magallana bilineata* is a vector for a number of oyster pathogens (Nuñal et al. 2023; Suja et al. 2020).

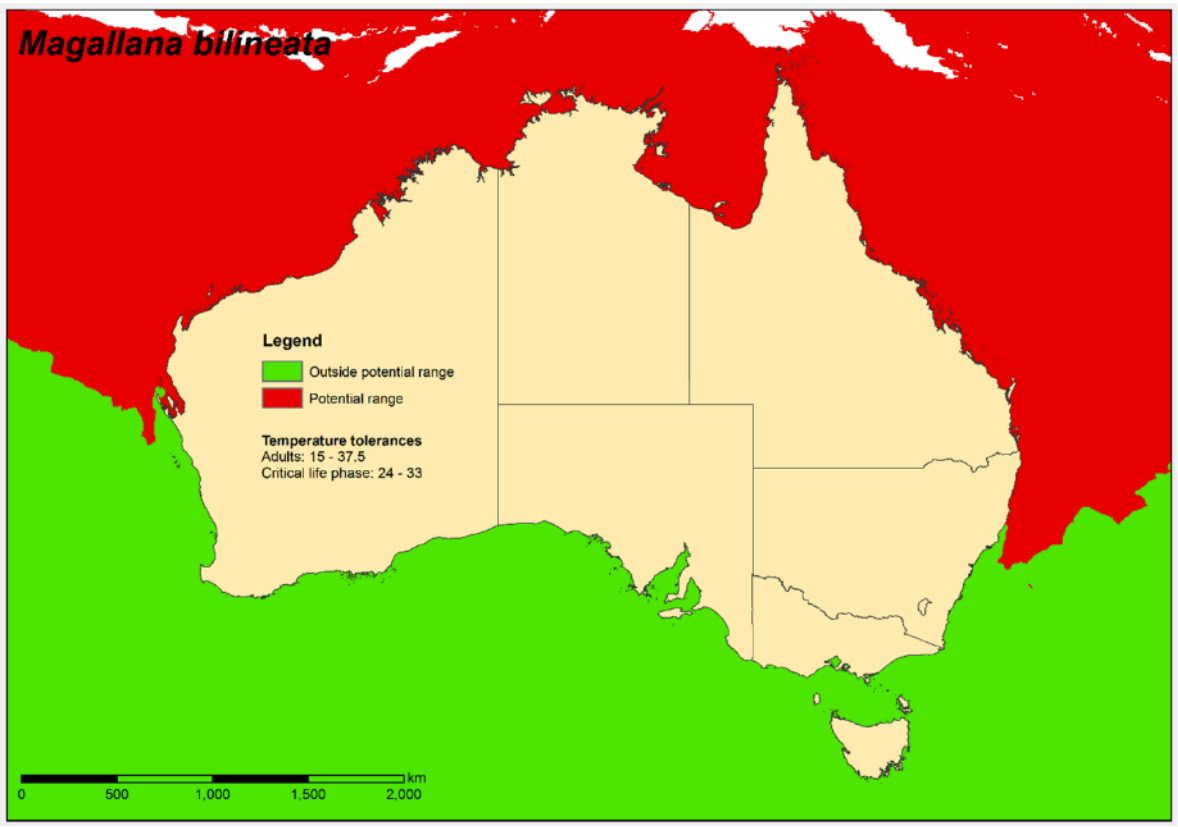
Global and Australian distribution

Magallana bilineata is native to the Indo-Pacific Ocean, with a wide distribution that spans Pakistan, India, Sri Lanka, China, Thailand, Malaysia, Indonesia, the Philippines, Japan, and Papua New Guinea (Willan et al. 2021). It is an important aquaculture species in its native range and has been introduced to Fiji (Kinch et al. 2019) and Australia (Willan et al. 2021). In Australia, established wild populations of *M. bilineata* occur in Far North Queensland, notably in Cairns, Port Douglas, Elim Beach, Cooktown, and Mourilyan Harbour (Map 21). It has previously been detected in Weipa, North Queensland, but the status of *M. bilineata* at Weipa is currently unknown. Species range mapping from ABARES shows that the northern half of Australia, from Shark Bay in WA to Port Macquarie in NSW, is potentially suitable for *M. bilineata* establishment (Map 22).

Map 21 Known global distribution of *Magallana bilineata*

Data source: GBIF.org (8 June 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.n9j3f4

Map 22 Maximum potential range of *Magallana bilineata* in Australian waters, indicating areas of potential suitability in red, and potential unsuitability in green



Data source: Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2021

Invasion history

In its native range in India and the Philippines, *M. bilineata* is an important aquaculture species. *Magallana bilineata* was deliberately introduced to Fiji in the 1970s for the purpose of aquaculture, where it is now established (Kinch et al. 2019). In 2019, it was detected in Far North Queensland, Australia, where it has now established. Hull biofouling is the most likely mechanism of translocation of the species in Australia (Willan et al. 2021).

Diseases

Suja et al. (2020) identified that wild and farmed *M. bilineata* populations in India are vectors for numerous oyster pathogens. Wild oysters were infected with protozoans (*Perkinsus beihaiensis*, *Nematopsis* sp., *Sphenophyra* sp., *Stegotricha* sp.), metazoans (cestodes and crustaceans), and shell parasites (*Polydora* spp. and *Cliona* spp.). Farmed *M. bilineata* populations exhibited fewer pathogens and pathological conditions than wild populations in India. Detections of pathogens such as *Escherichia coli*, *Vibrio* spp., and *Salmonella* spp. have occurred in *M. bilineata* farmed in the Philippines (Nuñal et al. 2023). These pathogens may impact human health when oysters are consumed.

Magallana gigas

*Magallana (*formerly *Crassostrea) gigas* is commonly called the Pacific oyster. *Magallana gigas* used to belong to the genus *Crassostrea* until it was placed into a new genus *Magallana* along with other Asia-Pacific cupped oysters (Salvi & Mariottini 2017). Records up until around 2017 will exclusively use the genus name *Crassostrea* (Willan 2021). Some current records still refer to *C. gigas* because of an opposition to the new genus *Magallana* (Bayne et al. 2017).

The species has been introduced to every continent except for Antarctica. It is native to Japan and south China. *Magallana gigas* dominates world bivalve aquaculture production and it is farmed throughout the world including Australia. *Magallana gigas* was introduced into Tasmania, South Australia, Victoria, and Western Australia in the mid-1900s. The oyster established in Tasmania and South Australia, is rare in Victoria, and did not establish in Western Australia. *Magallana gigas* was reported in New South Wales in the 1980s after it was suspected to have been introduced illegally. It is now established throughout the estuaries of New South Wales and is under movement controls to prevent further spread. It is also considered a pest in Tasmania and South Australia outside of aquaculture settings. *Magallana gigas* is affected by the ostreid herpesvirus-1 microvariant (OsHV-1 µvar) which can cause Pacific oyster mortality syndrome (POMS) disease, resulting in high mortality in affected populations.

Magallana gigas is not nationally listed in Australia on either the APMPL or the EEPL. It was excluded from the APMPL for being a widely cultivated species. Despite being widely cultivated in Australia, non-cultivated populations of *M. gigas* still pose threats to native bivalves and ecological communities (Herbert et al. 2016), and may have social, economic, or cultural impacts (Martínez-García et al. 2021).

Table 17 Taxonomic classification of *Magallana gigas*

Classification	*Magallana gigas*
Phylum	Mollusca
Class	Bivalvia
Order	Ostreodia
Family	Ostreidae
Genus	*Magallana*

Diagnostic features for identification

Field identification

Magallana gigas cannot be identified in the field due to high morphological variability, which makes differentiation between ostreid species challenging (Dridi et al. 2008). It cannot be consistently distinguished from *Saccostrea glomerata* in the field (Richard Willan [MAGNT], pers. comm., April 2023). Identification needs confirmation from an experienced taxonomist by assessing internal characters (particularly chomata) or through molecular diagnostics.

Magallana gigas have an elongated, irregularly shaped shell that can grow up to 450 mm long (Huber 2010), however, most specimens are around 80 to 150 mm. The valves are uneven in shape. The left valve is slightly convex whereas the right valve is deep, cup shaped, and overlaps with a frilled-folded appearance (Photo 27). Concentric lamellae are present in mature specimens. Wild *M.*

gigas found on Australian shores have morphologically very different appearances to cultivated *M. gigas.*

Photo 27 Adult *Magallana gigas* showing the wild, non-cultivated form

Source: Evan Rees, Northern Australia Quarantine Strategy (NAQS)

Similar native species

Magallana gigas can be difficult to distinguish from other oysters, including native oysters *Ostrea angasi*, *Saccostrea glomerata,* and *S. cucullata*. Ostreid species have shells that are highly variable in shape and colour, mainly due to the substrate where the spat settles (Poppe & Goto 1991). This variability makes identification challenging and molecular methods such as DNA barcoding can assist.

The congeneric *M. bilineata* has been recorded in northern Queensland and is considered an introduced pest. *Magallana gigas* can be distinguished from *M. bilineata* by the black muscle attachment scar present in the internal surface of both valves of *M. bilineata*. In 2023, the Suminoe oyster, *M. ariakensis,* was detected in Queensland in the Moreton Bay region and looks similar to *M. gigas* and *M. bilineata.*

Laboratory and molecular identification

A nested PCR for the detection of *Magallana gigas* was developed and demonstrated to be species-specific and able to detect *M. gigas* larvae added to plankton (Patil et al. 2005). This assay was modified and adapted to qPCR format by Bott and Giblot-Ducray (2012). Performance of the qPCR assay was validated using comparison with HTS testing in plankton samples from Australian areas with *M. gigas* populations (Wiltshire et al. 2019b). An alternative qPCR assay for *M. gigas* has been developed in Denmark (Andersen et al. 2018) but has not been tested under Australian conditions.

Refer to the guidelines for development and validation of assays for marine pests for further information and compendium of introduced marine pest molecular studies relevant to Australia.

Life history and ecology

Life habit

Magallana gigas is a fouling bivalve, capable of attaching to any hard surface in sheltered waters, usually rocks, but also marine infrastructure such as wharf pylons and boat ramps. They can also settle on the shells of other oyster species. *Magallana gigas* can settle in high numbers, up to 2000 oysters/m^2 and are quick growing (around 25 mm per year). The oyster lives in the intertidal or subtidal and is a suspension feeder.

Magallana gigas has broad environmental tolerances which is expected considering its geographic range of invasion, suggesting it is adaptable or tolerant of the local thermal conditions where it grows. In Maine, USA, *M. gigas* can experience water temperatures between –1 and 25°C (Shatkin 1997). The upper water temperature threshold for *M. gigas* is around 35°C (Mann et al. 1991), with mortality occurring within an hour at 40 and 43°C (Shamseldin et al. 1997). The optimal temperature range for growth is between 15 and 19°C.

Magallana gigas can also tolerate a wide range of salinities. The oysters have been reported from salinity as low as 3 ppt (Chu et al. 1996) with a similar salinity minimum reported from other studies (Mann et al. 1991). The salinity maximum for *M. gigas* is over 35 ppt. Freshwater and hypersaline treatments are unlikely to affect *M. gigas.* The salinity range of spat (recently settled juvenile oysters) is between 15 and 30 ppt.

Magallana gigas are predated on by several marine species in both their native and introduced ranges. Predators include seastars such as *Asterias* sp., boring gastropods, crabs, fish, and birds. *Magallana gigas* is severely affected by disease, in particular the ostreid herpesvirus-1 microvariant (OsHV-1 µvar).

Reproduction and growth

Magallana gigas are protandric hermaphrodites, starting off as a male and then changing sex as they grow. *Magallana gigas* are sexually mature after one year and adults can live up to 30 years (Nehring 2011). They grow at around 25 mm per year, reaching 10–15 cm after 2–4 years (Harris 2008). Spawning is temperature dependent, with breeding peaking in summer when the water is warmest (>20°C), with the optimum temperature between 20 to 25°C (Troost 2010). Spawning does not usually occur at water temperatures below 15°C.

Magallana gigas are highly fecund with the average female releasing between 50 to 200 million eggs in a single broadcast spawning event. Fertilisation occurs externally with larvae spending around 3 weeks in plankton (Chanley & Dinamani 1980). Spawning can occur in any salinity between 10 and 42 ppt, however, the optimal salinity range for spat is between 15 and 30 ppt (Shatkin 1997). The faster growth rate of *M. gigas* allows it to overgrow the native *Saccostrea glomerata* in lower tidal zones.

Pathways and vectors

Magallana gigas is a popular aquaculture species due to its fast growth rate. Because of this, *M. gigas* has been introduced throughout the world to establish aquaculture. *Magallana gigas* has

been introduced to North America, Europe, New Zealand, and Australia. As a common biofouling species, it can also be translocated across biogeographic areas via hull biofouling and biofouling of other mobile structures.

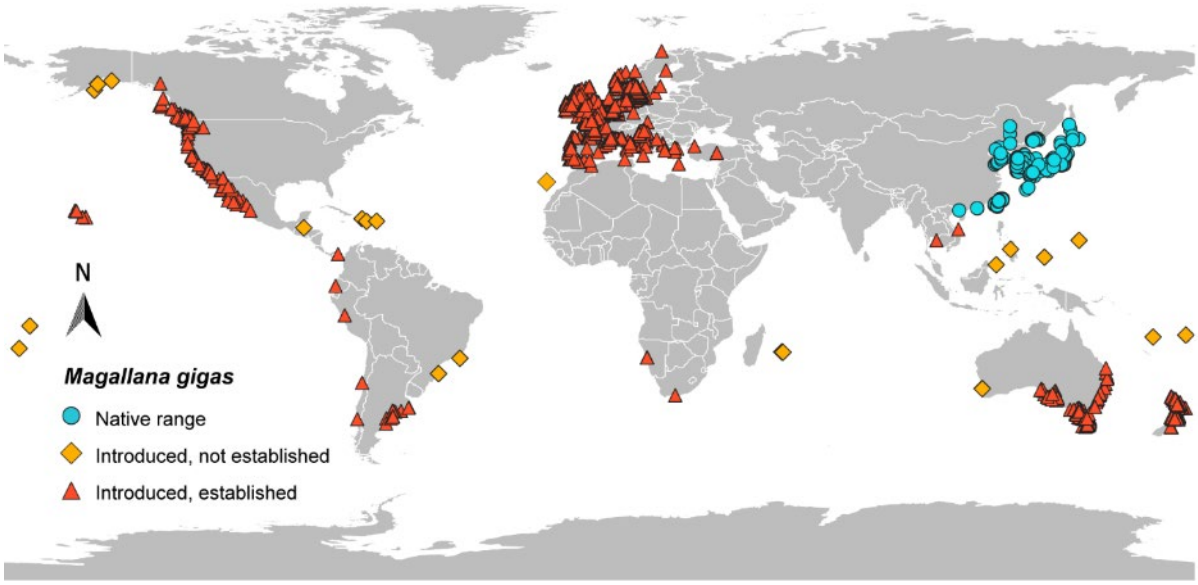
Potential impacts

Magallana gigas can settle in dense aggregations in the intertidal zone, limiting space and food for other intertidal species. A review by Herbert et al. (2016) found that non-native *M. gigas* reefs in Europe and the UK contribute to changes in ecological community structure in soft sediment and rocky intertidal habitats, with some communities displaying positive associations with *M. gigas*. In the Wadden Sea, high numbers of *M. gigas* prevented the recruitment and establishment of native mussels (Diederich 2006). *Magallana gigas* are also known to settle on shells of other oyster species, which create financial costs through increased cleaning and defouling in aquaculture settings. Keating et al. (2010) estimated an additional cost of up to $90,000 for *S. glomerata* farmers to manage *M. gigas*.

Global and Australian distribution

Magallana gigas is native to Japan and northern China and has been introduced to every continent, except for Antarctica, and has established in all major global oceans and seas (Map 23) (Padilla 2010). In Australia, established wild populations of *M. gigas* exist in Tasmania, South Australia, New South Wales, and less commonly in Victoria. *Magallana gigas* was intentionally introduced to southern Tasmania, Western Australia, and Victoria in the mid-1900s. It was found in the 1980s in New South Wales, suspected of having been introduced illegally.

Map 23 Known global distribution of *Magallana gigas*



Data source: GBIF.org (27 May 2024). GBIF Occurrence Download: https://doi.org/10.15468/dl.5xh47f

Invasion history

Magallana gigas is the most widely transplanted bivalve in the world and is the world's most cultivated oyster. Breeding populations have been established in the USA and Canada, Australia, New Zealand, Atlantic and Mediterranean Europe, South America, and South Africa. Common among *M. gigas* introductions is that it can go from a relatively confined aquaculture population to

becoming a major biomass component of wild systems. This process may take between three and ten decades (Krassoi et al. 2008). Because of this transition from aquaculture species to wild populations, it can be difficult to determine dates of introduction.

Magallana gigas was first introduced into Washington State, USA, in 1902 to bolster oyster stocks after the native *Ostrea lurida* had been overfished. *Magallana gigas* has subsequently spread along the Pacific coast of North America, where both farmed and wild populations exist. *Magallana gigas* was introduced into Chile in the 1970s (Castilla et al. 2005), where they mainly exist as aquaculture populations, with wild populations uncommon, possibly because of the water temperature. A failed aquaculture population led to the introduction and establishment of *M. gigas* in Patagonia, Argentina.

Magallana gigas was introduced into South Africa in 1950 (Robinson et al. 2005). The oyster was introduced into France in the 1960s following the decline of native oysters *Crassostrea angulata* and *Ostrea edulis*. From there, extensive plantings of *M. gigas* occurred throughout Atlantic Europe, including the United Kingdom, Ireland, Spain, Denmark, the Netherlands, and Germany. Around the same time, *M. gigas* was introduced into the Mediterranean Sea to replace declining native oyster stocks. *Magallana gigas* occurs through the Mediterranean from Morocco to Israel. A population of *M. gigas* also occurs at the mouth of the Baltic Sea.

Magallana gigas was introduced into Australia in the mid-1900s. It was initially introduced to Tasmania with imports from New Zealand in 1947 (Moloney et al. 2023). Commercial production of *M. gigas* began in Tasmania during the 1960s and then expanded into South Australia in the 1970s. It was introduced to Victoria and Western Australia at a similar time, and was later introduced into New South Wales in the early 1980s, where an established population of wild oysters now occurs (Moloney et al. 2023). Wild populations exist in South Australia, Tasmania, Victoria, and New South Wales and important aquaculture industries exist in South Australia, Tasmania, and New South Wales.

Numerous separate attempts have been made to introduce *M. gigas* into the tropics, including the Caribbean and the Pacific Islands. However, except for Hawaii, none of these introductions led to an established population.

Diseases

Aquaculture production of *M. gigas* is often characterised by high mortalities associated with ostreid herpesvirus-1 microvariant (OsHV-1 µvar*),* which causes Pacific oyster mortality syndrome (POMS) an acute disease in juvenile Pacific oysters, with up to 100% mortality in affected populations. POMS can lead to significant production loss for oyster farmers, employment, and business viability for cultivated *M. gigas*. There are concerns that wild *M. gigas* may be reservoirs of this pathogen that may pose risks to cultivated *M. gigas* farms.

OsHV-1 µvar was first detected in New South Wales in 2011 (Jenkins et al. 2013) at sites including the Georges River, Botany Bay, Parramatta River, Port Jackson, and later in the Hawkesbury River. OsHV-1 µvar was first recorded from Tasmania in 2016 (de Kantzow et al. 2017) and in wild *M. gigas* in Port Adelaide in 2018 (PIRSA 2019). Other herpesviruses have been reported from *M. gigas* but have not been associated with as severe disease as with OsHV-1 µvar (Hine et al. 1992).

The development of POMS caused by the virus OsHV-1 µvar is related to water temperature. Under experimental conditions de Kantzow et al. (2016) demonstrated that cumulative mortality of *M. gigas* from POMS was 100% at water temperatures 22 and 24°C and significantly less mortality was observed at 18 and 20°C with no mortality observed at 14°C.

Translocation of OsHV-1 µvar by *M. gigas* under experimental conditions mimicking biofouling assemblages only occurred for one of eight replicates when *M. gigas* was the infected species (Fuhrmann et al. 2021). This was evidence of an empirically demonstrable pathway for spread of pathogens via biofouling containing *M. gigas*, albeit only a rare instance.

An AQUAVETPLAN manual exists for OsHV-1 µvar and POMS that includes information on the pathobiology, epidemiology, diagnostic methods, and methods to control and eradicate the pathogen in Australia (DAFF 2015).

The parasite *Haplosporidium nelsoni* was reported to have been introduced into the Chesapeake Bay with infected *M. giga*s (Stokes & Burreson 1995). *Haplosporidium nelsoni* is also known as multinucleated sphere unknown (MSX) and following its introduction, it significantly impacted farmed and wild populations of the native American oyster, *Crassostrea virginica*. In contrast, movement of *M. gigas* to France from the USA resulted in the co-introduction of *H. nelsoni,* but no consequences have been identified to-date.

Magallana gigas is known to be affected by several other diseases. The parasitic copepod, *Mytilicola orientalis,* has spread throughout Europe associated with movements of *M. gigas*. The copepod has impacted native species such as *Mytilus edilus*, *Cerastoderma edule* and *Macomoa balthica* (Goedknegt et al. 2020). A fungus, *Ostracoblade implexa,* has also been spread into new areas of Europe because of *M. gigas* movements (Blakeslee et al. 2013).

Bonamia-like parasites have been observed in *M. gigas* and DNA sequences matching *B. ostreae* have been reported from *M. gigas* (Diggles 2003; Lynch et al. 2010). No confirmatory diagnosis of *Bonamia* sp. has been made from *M. gigas*.

Magallana gigas can be infected by bacteria from the *Vibrio* genus. These infections can cause mass mortalities in cultivated *M. gigas* (Coyle et al. 2023), and several *Vibrio* spp. can cause infections in humans.

Appendix B: Policy principles for determining the current status of marine pests

The policy principles provide a flexible approach to determining current pest status of marine pests and in the absence of agreed surveillance approaches (currently under development), general policy principles should be applied, rather than adopting a prescriptive policy. General policy principles that have been identified include:

- For incidents where the Consultative Committee on Introduced Marine Pest Emergencies (CCIMPE) convenes and provides advice to the National Management Group (NMG), CCIMPE will recommend processes to determine pest status (e.g. likely present, likely absent, or unknown) and propose a pest status confidence level on a case-by-case basis.
- For incidents that are not referred to the NMG, the combat jurisdiction decides on processes to determine pest status. CCIMPE may still provide non-binding advice as part of this decision.
- In scenarios not related to specific pest incursions (e.g. aquaculture site selection), jurisdictions will make the determination of presence (including range)/absence of a pest within their jurisdictional waters.
- It should be noted that pest status is valid as of the time of most recent determination but subject to change due to on-going introduction risk over time. Pest status determinations may therefore need to be repeated, with frequency dependent on the risk of introduction.
- Surveillance methods used in determining pest status should be recorded and shared upon request.
- Both quantitative and qualitative determinations of pest status can be used, as appropriate for the marine pest, location, and conditions.
- Methods used should be appropriate for the target species; pest biology should be considered with respect to surveillance duration, timing, and sampling method.
- For quantitative determinations, quality assurance data are required for method accuracy as applied to the relevant situation (target species, habitat type etc.). Specifically, quantifying current pest status requires, amongst other things, knowledge of the likelihood of false negatives (failure to detect pests when present) and of false positives (apparent detection of species that are not present).[6]

[6] Epitools offers several tools to assist in decision making for sampling numbers and is freely available and easy to use. The South Australian Research and Development Institute (SARDI) has developed a sample number calculator for surveillance using plankton samples tested with quantitative polymerase chain reaction (qPCR) assays (Survey Sample Number Calculator). Both tools require target survey confidence and minimum abundance to be specified, and estimates of test performance provided to calculate the number of samples required.

  - For eDNA approaches, the performance of both sampling collection methods and of the molecular tests applied need to be understood to provide quantitative determinations. Effects of sample timing on likelihood of detection should also be considered.
  - Qualitative determination can be made where the method has been appropriately demonstrated but its performance has yet to be quantified, e.g. eDNA methods that have demonstrated detections in appropriate sample types but where the specific likelihood of false negatives and false positives is unknown.
  - Methods that allow quantitative determination should be applied in preference where feasible.
  - Pest status cannot be determined with any confidence if methods have not been validated or are inappropriate for the circumstance.
- Management implications should be considered, and caution applied when making pest status determinations because the level of confidence in presence or absence will depend on the extent and effectiveness of surveillance methods used in determining pest status.

Appendix C: Using the *Biosecurity Act 2015* during an emergency response

The following is an interim process for using the *Biosecurity Act 2015* (the Act) for action on vessels to treat contaminations by a marine pest of national significance. The *Biosecurity Act 2015* may be used in certain circumstances, including where a biosecurity officer suspects on reasonable grounds, that the level of biosecurity risk associated with the vessel is unacceptable. Under these circumstances, a biosecurity officer may, in relation to a vessel that is under biosecurity control, direct:

- the person in charge or operator of a vessel not to move, interfere with, or deal with the vessel
- the person in charge or operator of a vessel to move the vessel to a specified place, including a place outside of Australian territory
- a vessel to undergo treatment action deemed necessary by the biosecurity officer
- that other biosecurity measures which may be prescribed by regulations be undertaken.

In addition, biosecurity officers may exercise certain powers, such as taking samples of ballast water from vessels, for the purpose of monitoring compliance with provisions for the management of ballast water at a port or offshore terminal within the outer limits of the exclusive economic zone of Australia. Where the Director of Biosecurity (or delegate) is satisfied that a sample of the vessel's ballast water indicates that the vessel poses an unacceptable level of biosecurity risk, then the Director may give a direction to the vessel not to discharge ballast water until conditions specified in the direction are met.

The conditions of using the *Biosecurity Act 2015* are:

- the Australian Government Department of Agriculture, Fisheries and Forestry is to be contacted before taking the proposed action to determine the appropriate provisions of the *Biosecurity Act 2015* that apply
- directions to take action under the *Biosecurity Act 2015* are to be given by a biosecurity officer. Officers of a state or territory government must be authorised as biosecurity officers under the *Biosecurity Act 2015* to be able to give directions under the Act
- actions under the *Biosecurity Act 2015* should only be taken for vessels currently identified as at a risk of spreading a marine pest of national significance.

Responsibility for directing and approving action under the *Biosecurity Act 2015* rests with the biosecurity officer, but the actual vessel control and treatment actions are handled by the Local or State Control Centre. As a matter of policy, the following information should be provided to the Australian Government Department of Agriculture, Fisheries and Forestry to help determine appropriate application of the *Biosecurity Act 2015:*

- the proposed course of action
- the location of proposed action

- details to identify the vessel involved in the proposed action
- contact details of local management agencies that will be managing the vessel control and treatment.

Appendix D: Commonwealth, state, and territory legislative powers of intervention and enforcement

The Intergovernmental Agreement on Biosecurity (IGAB) is an agreement between the Commonwealth, state, and territory governments. It came into effect in January 2019 and replaced the previous IGAB which commenced in 2012. The agreement was developed to improve the national biosecurity system by identifying the roles and responsibilities of governments and outlining the priority areas for collaboration to minimise the impact of pests and disease on Australia's economy, environment, and community. The National Environmental Biosecurity Response Agreement 2.0 was the first deliverable of the IGAB and sets out emergency response arrangements, including cost-sharing arrangements, for responding to biosecurity incidents primarily affecting the environment and/or social amenity and when the response is for the public good. In combination with the IGAB, Commonwealth, state, and territory governments are responsible under their principal fisheries management legislation to respond consistently and cost-effectively to a marine pest incursion (Table 18).

Table 18 Commonwealth, state, and territory legislation covering emergency response arrangements

Jurisdiction	Agency	Principle fisheries management acts covering emergency response arrangements	Relevant contact website
Commonwealth	Department of Agriculture, Fisheries and Forestry	*Biosecurity Act 2015* *Fisheries Management Act 1991*	https://www.marinepests.gov.au/report agriculture.gov.au/biosecurity-trade/pests-diseases-weeds/marine-pests
New South Wales	Department of Primary Industries and Regional Development	*NSW Biosecurity Order (Permitted Activities) 2019* *NSW Biosecurity Regulation 2017* *NSW Biosecurity Act 2015* *Fisheries Management (General) Biosecurity Regulation 2017* *Fisheries Management (Aquaculture) Regulation 2012* *Fisheries Management Act 1995* *Marine Safety Act 1998* *Marine Parks Regulation 1997* *Ports and Maritime Administration Act 1995*	dpi.nsw.gov.au/fishing/pests-diseases
Victoria	Department of Energy, Environment and Climate Action	*Marine and Coastal Act 2018* *Marine Safety Act 2010* *Fisheries Act 1995* *Port Management Act 1995* *Environment Protection Act 1970*	vic.gov.au/marine-pests

Jurisdiction	Agency	Principle fisheries management acts covering emergency response arrangements	Relevant contact website
Queensland	Department of Primary Industries	*Biosecurity Act 2014* *Fisheries Act 1994*	daff.qld.gov.au/fisheries/ qld.gov.au/environment/coasts-waterways/marine-pests
South Australia	Department of Primary Industries and Regions, South Australia	*Fisheries Management Act 2007*	pir.sa.gov.au/biosecurity/aquatics
Western Australia	Department of Primary Industries and Regional Development	*Biosecurity and Agriculture Management Act 2007* *Fish Resources Management Act 1994*	fish.wa.gov.au/Sustainability-and-Environment/Aquatic-Biosecurity/Pages/default.aspx
Tasmania	Department of Natural Resources and Environment Tasmania	*Biosecurity Act 2019* *Living Marine Resources Management Act 1995*	nre.tas.gov.au/biosecurity-tasmania/aquatic-pests-and-diseases
Northern Territory	Department of Agriculture and Fisheries	*Fisheries Act 1988*	nt.gov.au/marine/for-all-harbour-and-boat-users/biosecurity/aquatic-pests-marine-and-freshwater

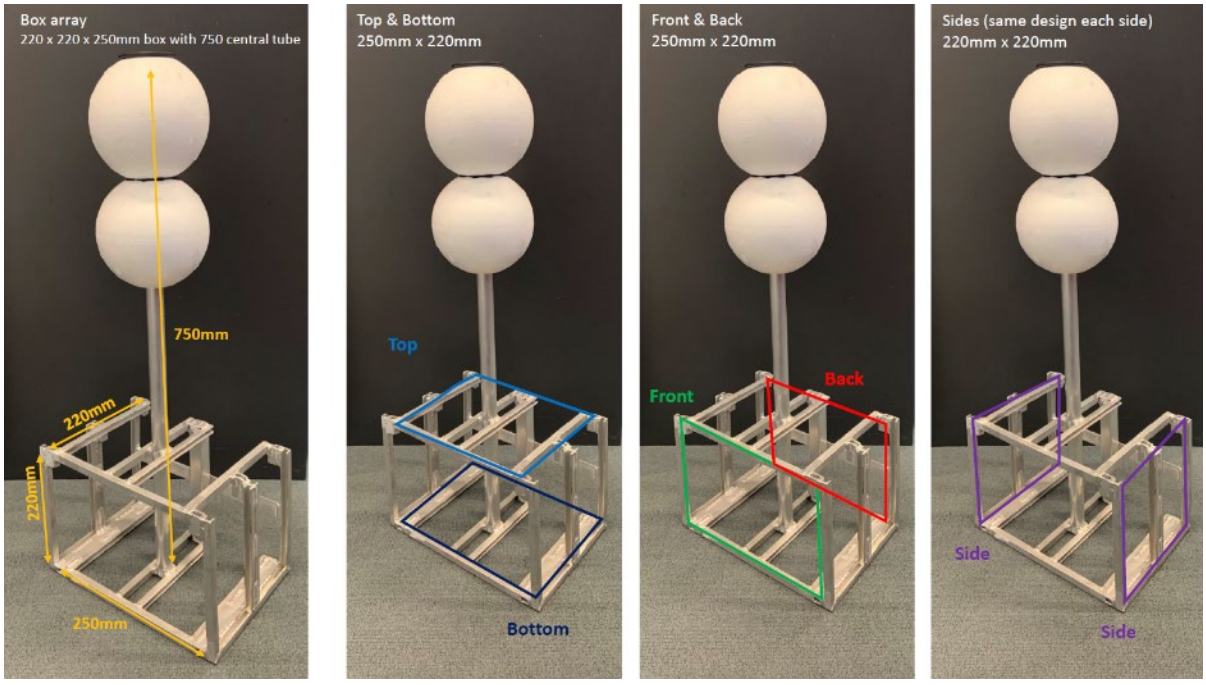
Appendix E: Settlement array designs to sample invasive marine bivalves

Settlement arrays consisting of settlement plates held in different configurations are commonly used to study recruitment of sessile and fouling marine organisms during marine pest surveillance and monitoring programs. This appendix covers some settlement array designs used for sampling invasive marine bivalves among other sessile marine organisms. See page 106 in the Australian marine pest monitoring manual for details on sample processing methods for settlement array samples.

Box array

Several jurisdictions in Australia now routinely use a box-array design for invasive marine species surveillance. The box array was developed by Aquatic Pest Biosecurity, Western Australian Department of Primary Industries and Regional Development (WA DPIRD). The box array is made with an aluminium frame, which will need to be constructed by an aluminium fabricator. Two designs are available for the box array, consisting of a 'standard' and a 'stepped out' box array design. The stepped-out design contains additional spacing between railings which reduces the amount of biofouling that is accidentally scraped off when removing the plates (Photo 28).

Photo 28 Stepped box array design showing different sides and measurements

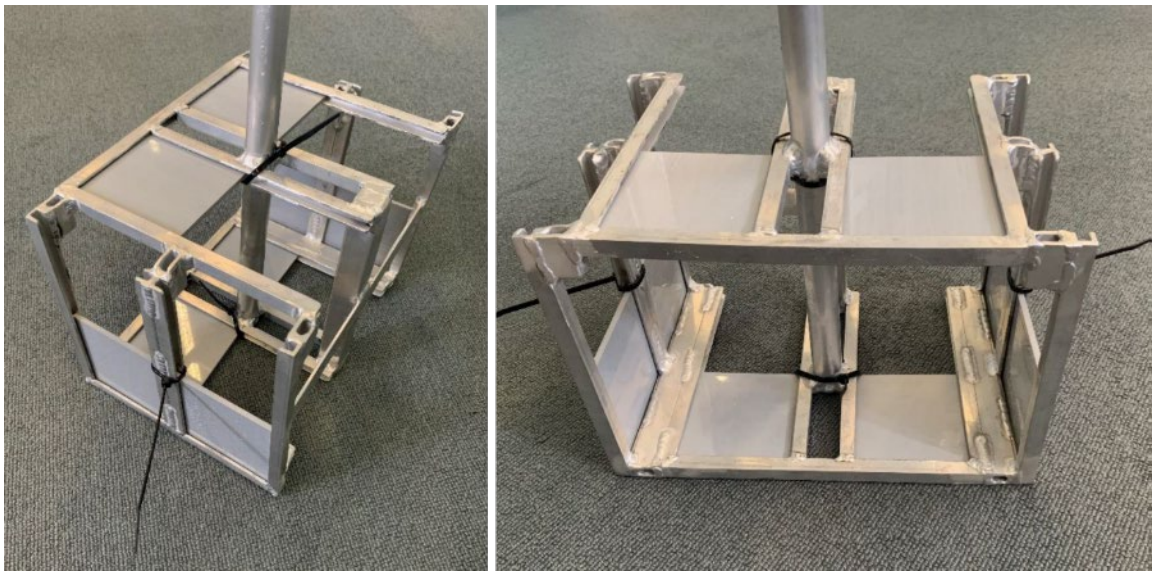


Source: Aquatic Pest Biosecurity, WA Department of Primary Industries and Regional Development

The box arrays are designed to hold square plastic polyvinyl chloride (PVC) settlement plates measuring 100 x 100 mm, and at 4.5 mm thick (Photo 29). The PVC plates are the substrate for which settlement of sessile marine organisms occur. These PVC plates are scuffed to create a rough surface texture which promotes the fouling of organisms. The plates are designed to slide in and out

of designated 'U-channels' in the box arrays. A box array typically holds a total of eight plates per deployment but can also hold an additional eight spare plates if needed (16 plates total). The PVC plates can be reused in subsequent deployment as long as they are sufficiently cleaned and decontaminated.

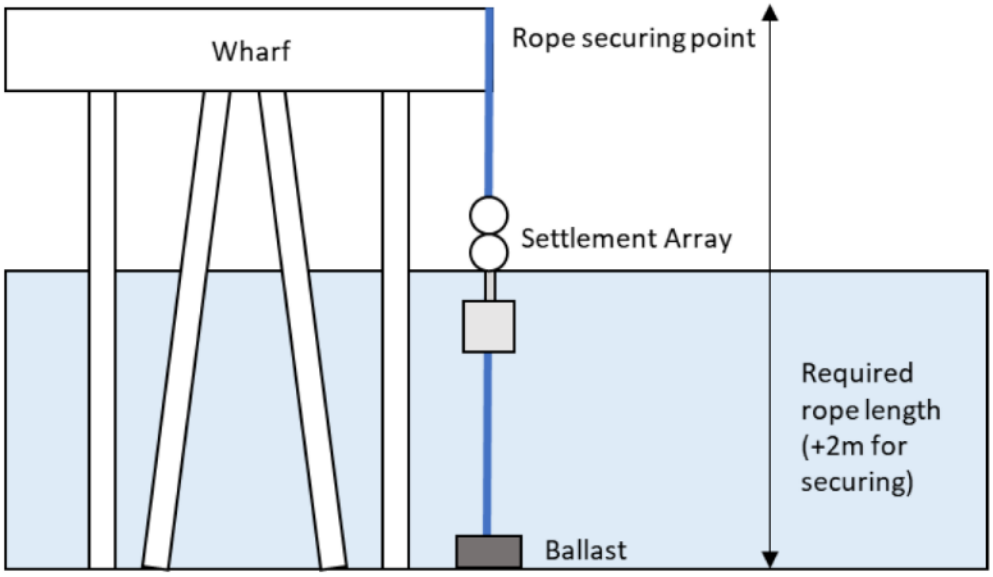
Photo 29 Grey PVC plates (10 cm^2) inserted into U-channels of the box array



Source: Aquatic Pest Biosecurity, WA Department of Primary Industries and Regional Development

Box arrays are fitted with two 150 mm diameter floats to provide buoyancy to the array, which are designed to float at the surface so that the plates sit at a constant depth of ~1 m below the surface (Figure 8). Float savers are fitted to reduce rope friction. Marine-grade rope is used for attaching the array to a wharf, pontoon, or other similar stricture. A ballast (minimum 4 kg) is used to anchor the settlement array rope to the sea floor which prevents the array from drifting.

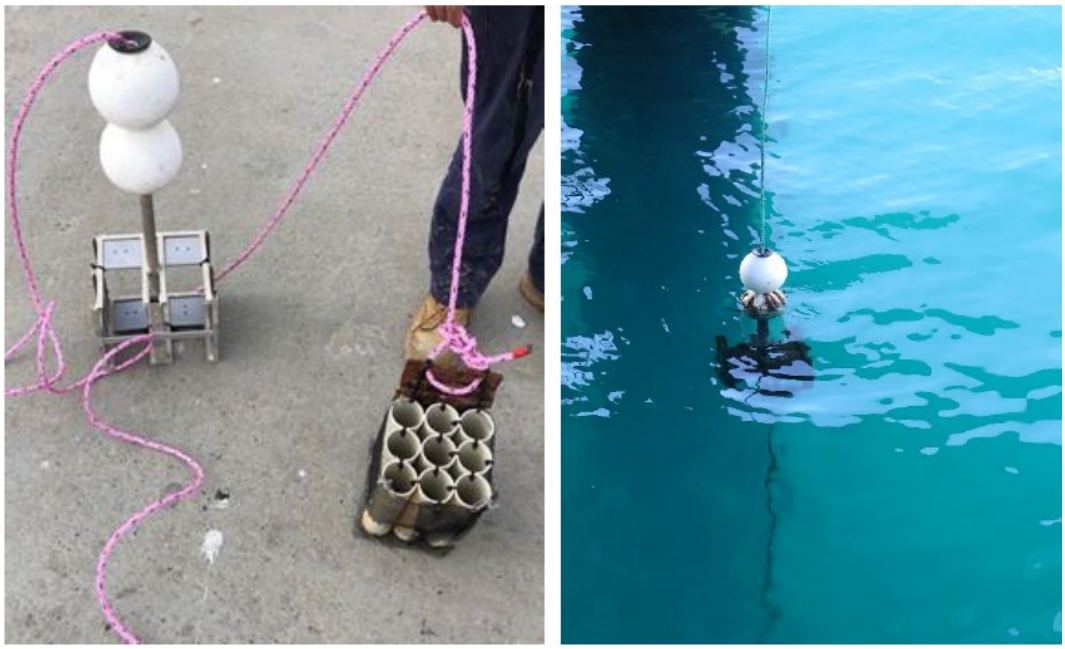
Figure 8 Schematic of typical box settlement array deployment



Source: Aquatic Pest Biosecurity, WA Department of Primary Industries and Regional Development

Box arrays are deployed and their position marked, and additional crab condos may be added to the array (Photo 30). The length of time a box array is deployed varies, but usually arrays are deployed for two months at a time. Arrays may be deployed twice a year to capture summer and winter seasonal peaks, or more frequently (e.g. four or six times a year). Multiple box arrays may be deployed at a site at a given time.

Photo 30 Box settlement array and crab condo ready for deployment (left) and box array deployed in-water (right)

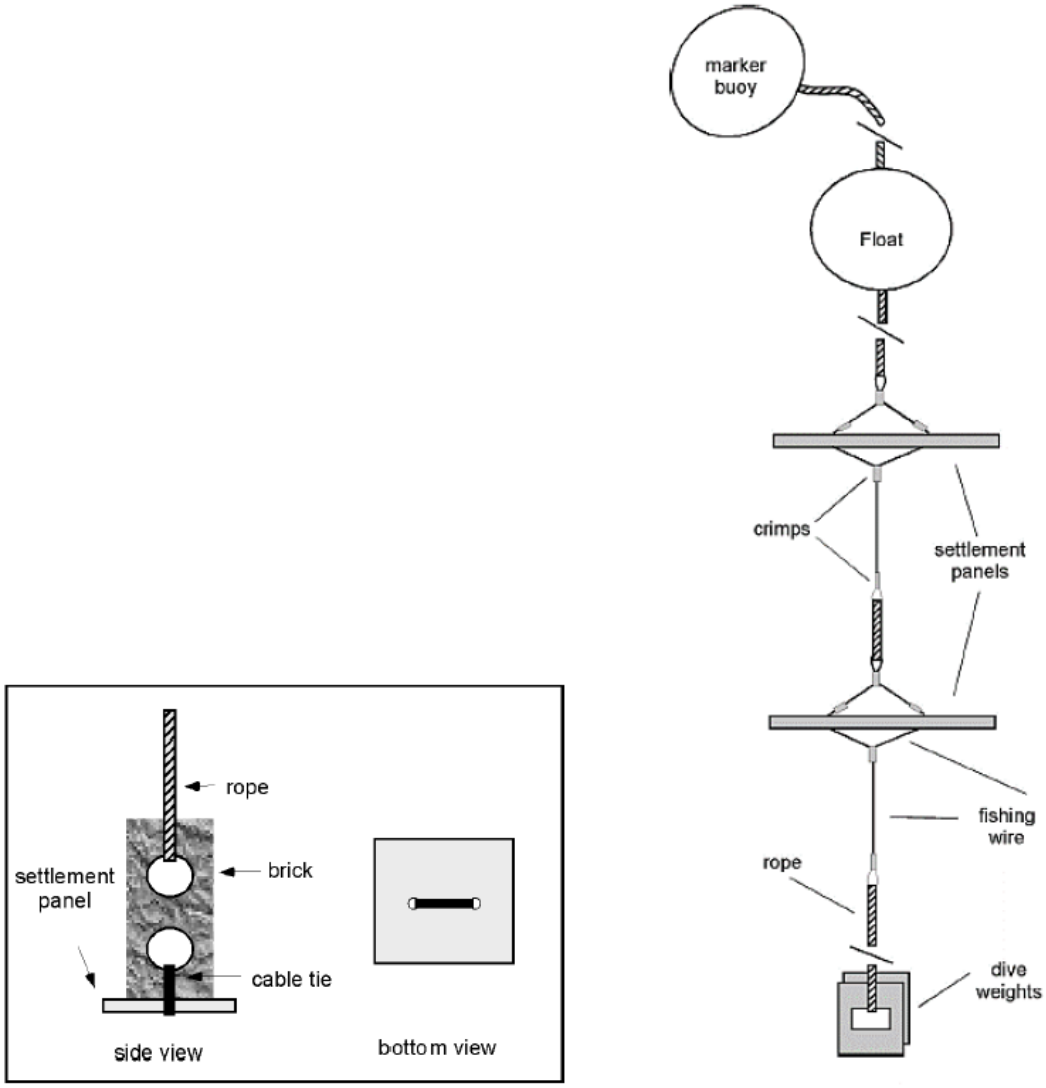


Source: Aquatic Pest Biosecurity, WA Department of Primary Industries and Regional Development

Hanging settlement array

The 'hanging' settlement array is constructed with PVC plates deployed at ~2 m below the surface (Sutton & Hewitt 2004) (Figure 9). Generally, the design uses 14.5 cm x 14.5 cm PVC plates that are abraded by sandblasting on one side. Two holes are drilled in the middle of each plate and secured to a brick with cable ties with the roughened side facing away from the brick. One end of the rope is attached to the brick and the other to a structure in the environment, for example a wharf piling. Sutton and Hewitt (2004) recommended securing the plates horizontally at a depth of 2 m below the low tide. The plates should be deployed for a minimum of three months to allow biofouling to reach a size and maturity to enable high taxonomic resolution.

Figure 9 Hanging settlement design recommended by Sutton and Hewitt (2004)



Source: Tait and Inglis (2016)

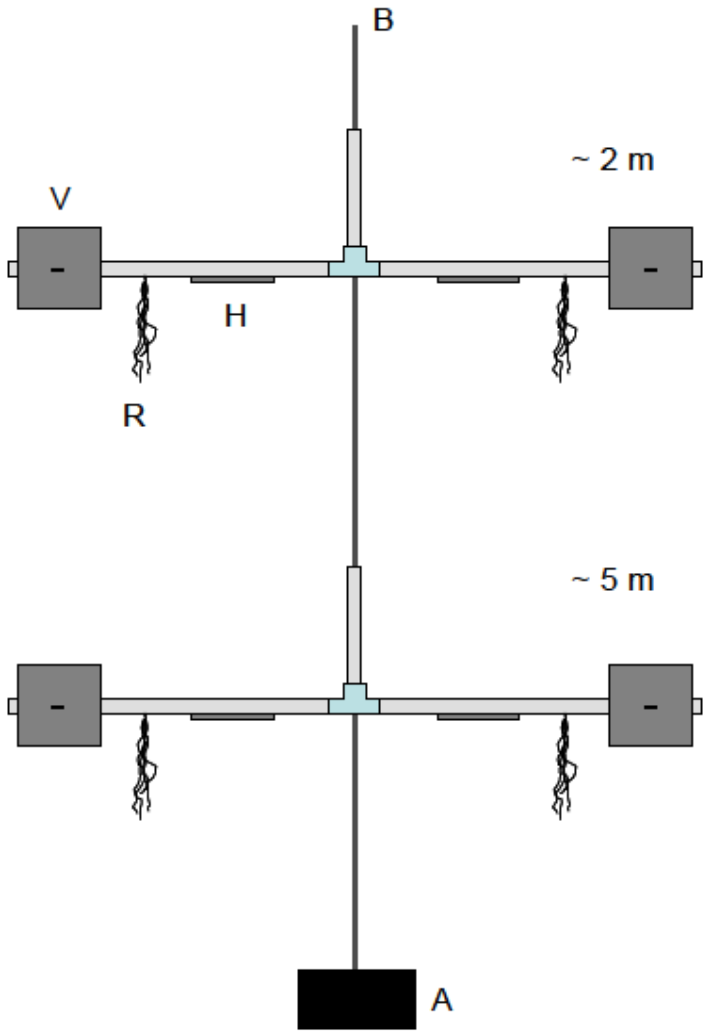
Double T-unit array

A monitoring programme for marine biofouling organisms in 2000 followed the incursion and eradication of *Mytilopsis sallei* in the Northern Territory (Cribb et al. 2010). The programme principally targeted *Mytilopsis sallei, Perna viridis,* and *Arcuatula senhousia*. The original settlement plate design (Figure 10) consisted of a rope backbone to which two PVC pipe T-units were secured. The T-units were attached to the rope backbone at two water depths at ~1 m below the water surface and ~1 m above the seafloor (Ferguson 2000). Each T-unit has two horizontal arms comprising 0.7 m lengths of 25 mm diameter PVC pipe. This array is called the 'double T-unit array' for the purpose of this manual.

On each T-unit, two 14.5 x 14.5 cm flat sheets of PVC were fixed horizontally and two were fixed in a vertical potion to target organisms with different light requirements (not particularly relevant for marine bivalves). A 15 cm length of 'hairy' or 'Christmas tree' rope mop was suspended midway along each horizontal tube. The arrays were deployed by attaching the rope backbone to the

undersides of wharves using a metal eyelet drilled into the wharf. The base of the array was anchored to the seafloor by a single concrete block. Variations of this design included different depths of deployment and number and orientations of PVC plates.

Figure 10 Double T-unit array design showing vertical plates (V) and horizontal plates (H)



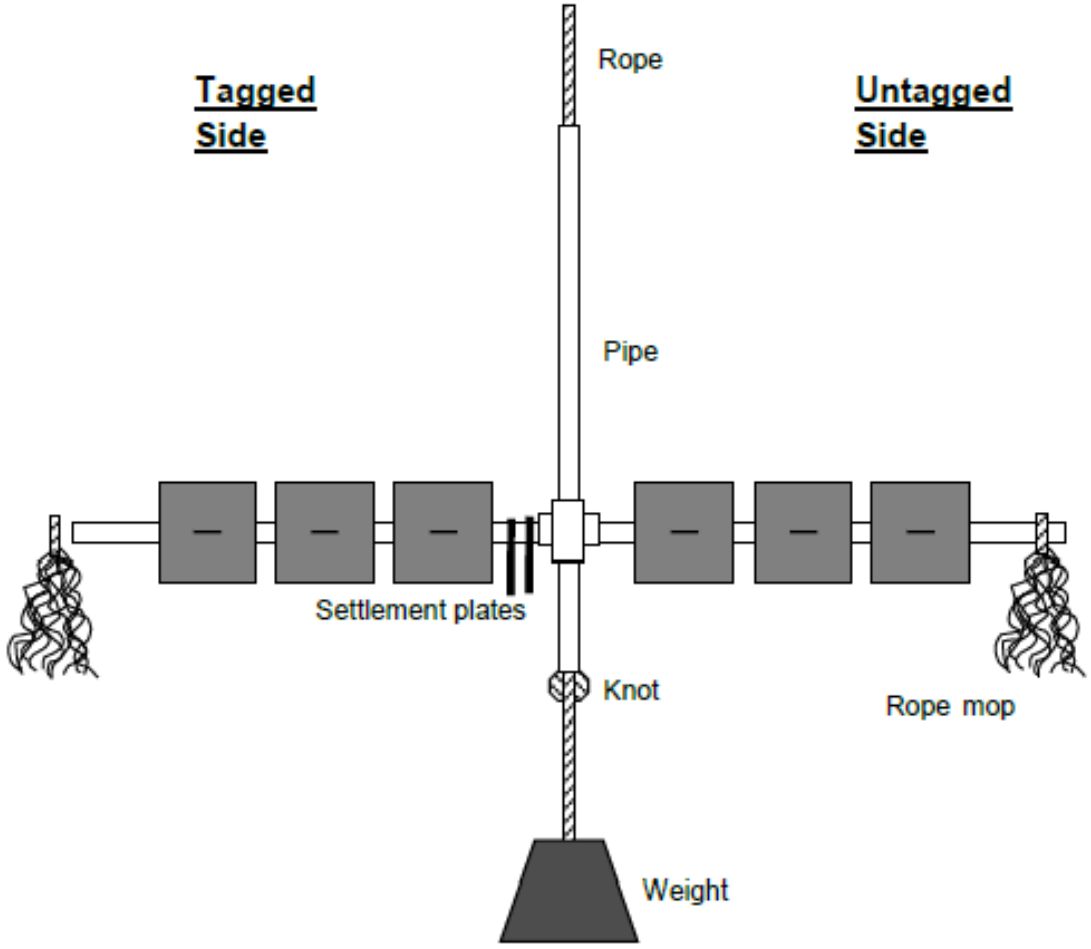
Source*:* Floerl et al. (2012)

Single T-unit array

One variation of the double T-unit design has been used in the Northern Territory (Figure 11), which has been called the 'single T-unit array' for the purpose of this manual. This array is suspended from a floating structure, such as a mooring buoy or pontoon, so that its moves vertically with the tide and the settlement surfaces are maintained at around 2.5 m depth. This design includes deploying three PVC plates and a single rope mop on one arm of the T-unit (the 'tagged' side). On initial setup plates and rope mop are installed on one side, and at the subsequent three-monthly inspection a second set is installed on the second side. Plates and mops are collected for lab examination after six months, and field notes made on the other array that is left in the water. Tagging one side ensures

that the correct side is collected as sometimes it can be difficult to distinguish plates with three months of fouling from those with six months fouling.

Figure 11 Single T-unit array design



Source: Northern Territory Government (2014)

Other array designs

Other settlement arrays have been designed and tested for invasive marine species monitoring, such as a frame array designed by Tait et al. (2016) in New Zealand. As described in a literature review by Tait and Inglis (2016), there are various variables to consider when designing and deploying settlement arrays. The box array is becoming the more popular design used in invasive marine species surveillance programs across Australia.

Appendix F: Using plankton and water samples to detect bivalve larvae, gametes and eDNA

This appendix provides an example method for collecting and preserving plankton and water samples to detect and quantify bivalve larvae, which can also be used for eDNA surveillance. Further detail on plankton and water samples, as well as other methods for marine pest surveillance, are located in the Australian marine pest monitoring manual. The marine pest monitoring manual contains information on sample handling and preferred narcotising, fixation, and preservation techniques for major taxonomic groups including bivalves. It also gives advice on appropriate levels of experience required for sample processing for plankton tows and other methods.

Plankton samples can be collected using a 50–70 cm diameter bongo or conical plankton net with cod end. Mesh size of the net varies but usually 50–150 µm mesh is recommended for marine pest surveillance. The mesh size is selected based on the target life stage size (e.g. bivalve larvae) noting that finer mesh can be prone to clogging in environments with high planktonic abundance or suspended sediments. The net is towed behind a vessel obliquely from the sea floor (if shallower than 10 m depth) to the water surface, but can also be towed vertically or horizontally. Tow distance varies, but usually occurs over 100–300 m transects at a set speed (e.g. 1–1.5 knots), depending on the biomass obtained in the samples, the location, and the surveillance program design. Occasionally tow duration may be used instead of distance. A mechanical flow meter should be fitted to the net frame and used to estimate the volume of water filtered for each tow. After each deployment, the net should be rinsed and the sample from each net washed in separate small mesh (30–100 µm) net sieves to remove as much seawater as possible.

Alternatively, plankton samples may be obtained using a centrifugal, motor-driven pump with a throughput of about 0.5 m^3/minute (Queiroga et al. 1994). Pump output should be measured and kept approximately constant for all samples. Samples should be taken throughout the top 20 m of the water column at 1 m depth intervals or greater, but no closer than 0.5 m from the bottom. Water retrieved by the pump should be passed through a fine mesh net to retain the larvae. The mesh size is selected based on the target plankton size. After each deployment, the net should be rinsed using a bilge pump and the sample from each net washed in separate small 100 µm mesh net sieves to remove as much seawater as possible.

Water samples can be collected using buckets or bottles, including Niskin bottles to collect at depth. Water samples need to be filtered following collection. Some jurisdictions, like Victoria and Queensland, use eDNA water samplers with self-preserving filters in their marine pest surveillance programs (see Thomas et al. 2018).

If both morphological and molecular approaches for plankton identification are used, net samples should be subsampled and processed separately for microscopic identification and molecular diagnostics. See page 114 in the Australian marine pest monitoring manual for details on sample processing methods for invertebrate larvae net tows.

Plankton samples that will be analysed using molecular diagnostics should not be put into formalin, as formalin vapor can deteriorate the DNA. Instead, these samples should be rinsed into sample jars with SET-buffered, reagent-grade ethanol (usually 70% or 90%), ensuring that the ratio of biomass to SET buffered ethanol is no more than 1 to 3. Nucleic acid preservation buffers can also be used, e.g. RNAlater® or Longmires solution, providing these are compatible with the DNA extraction method to be applied.

Each sample should be labelled with:

- details of the location in which it was collected (including latitude and longitude where possible)
- the method used to collect the sample (e.g. plankton tow or pump)
- the sample identifier (such as number in sequence of samples or sample code)
- the date and time collected
- the name of the collector.

Additional information collected with the sample (such as environmental variables, tow speed and duration, depth of collection) should be recorded separately and should also include details of the date of collection, the sample identifier, the method used and location details.

Glossary

Term	Definition
Active surveillance	Biosecurity surveillance carried out in a fully structured way, such as according to formal protocols in a specified surveillance program, usually undertaken by paid staff from government or industry agencies.
Aquatic species	Any organism which spends all or significant parts of its lifecycle in fresh, brackish, or marine waters.
Ballast water	Water with its suspended matter (i.e. sediment) taken on board a vessel into its ballast tanks or cargo holds to control trim, list, draught, stability, or stresses of the vessel.
Biofouling	Biofouling is the attachment or accumulation of aquatic organisms such as microorganisms, plants, and animals, to any part of a vessel, or on surfaces and structures immersed in or exposed to the aquatic environment. Biofouling is also known as hull fouling.
Biological control	Control of pests and weeds by another organism (e.g. insect, bacteria, virus etc), by a biological product (hormone), or by genetic or sterility manipulations.
Biosecurity	Managing risks to Australia's economy, environment, and community from pests and diseases entering, emerging, establishing, or spreading to or within Australia
Bivalve	Invertebrate animals of the class Bivalvia. Bivalves are aquatic molluscs that have laterally compressed bodies enclosed by a shell consisting of two hinged parts, or 'valves'. Common bivalves include oysters, mussels, clams, cockles, scallops, and false-mussels.
Containment	The application of measures in and around an infested area to restrict the spread of an invasive pest to a defined region. This may include reduction of the density or area of the infestation where appropriate or managing vectors. A containment program may include eradication of satellite infestations.
Control	In relation to organisms, control actions are those which aim to reduce the number of pest organisms, prevent an increase in pest numbers and spread, reduce organism activity to limit their impact, or modify the behaviour or characteristics of the pest population. Control may involve partial eradication or other actions which limit population size and/or reproductive potential. This term is sometimes used interchangeably with 'management.'
Cost benefit analysis	A comparative analysis of all costs and benefits of undertaking different options, to help decide which actions provide the best value or most suitable outcome (may include the 'do nothing' option).
Cryptogenic species	A species of obscure or unknown origin for which it is not possible to reliably determine whether they are introduced or native.
Decontamination	Decontamination is the cleaning or treatment of material used to remove marine pests or render marine pests non-viable, including their propagules and any parasite and pathogen that can be associated with the marine pest species.
Delimitation	Delimitation establishes the geographic extent of an area infested by, or free from, a marine pest, and specifically informs feasibility of eradication or areas to target for control and management.
Destruction	The process of killing aquatic organisms for eradication or control purposes.
Endemic species	A species with a native distribution restricted to the bioregion(s) of interest.
Eradication	Under the NEBRA, eradication in relation to pests means eliminating the pest from an area. Eradication is indicated by the pest no longer being detectable.
Established marine pest	A self-sustaining pest that occurs in Australia and is regarded as not eradicable. An established pest may be distributed widely across Australia or be only regionally distributed. A regionally-distributed established pest may be the subject of containment

Term	Definition
	measures to mitigate further spread. Native or indigenous plants and animals are not characterised as established marine pest (even if having negative impacts).
Fouling organism	Any plant or animal that attaches to natural and artificial substrates such as piers, navigation buoys, pilings, or hulls. Includes crawling and nestling forms as well as seaweeds, hydroids, barnacles, mussels, bryozoans etc.
General surveillance	General surveillance activities which are not specifically focused on a single or select number of marine pest species. General surveillance activities have one or more element(s) of opportunism, on a spectrum ranging from fortuitous *ad hoc* detections to relatively highly structured activities but excludes active surveillance. An example is a report of an unusual organism by a member of the public.
Hazard	A situation/activity that under certain conditions will cause harm. The likelihood of these conditions and magnitude of the harm produces a level of risk.
Incursion	Occurrence of an introduced species in a region or country where it is not already established. See Interception.
Infaunal	Organisms living within substrate (e.g. burrowing species).
Infestation/infested area	Population, or area with a population, of the introduced species.
Interception	Detection of a non-native organism at a pre-border or border inspection point, quarantine facility or other type of biosecurity control location.
Invasive marine species	See 'marine pest'.
Management	Actions taken in response to an introduced species including surveillance, control, containment, destruction etc.
Marine pest	Non-native marine plants or animals that may harm Australia's marine environment, social amenity, or industries that use the marine environment, or species that have the potential to do so if they were to be introduced, established (i.e. forming self-sustaining populations) or spread in Australia's marine environment. Many terms are used, sometimes interchangeably, to describe plants and animals that have been moved beyond their native range, including alien, exotic, introduced, invasive, non-indigenous, non-native and nuisance species.
Marine species	Any aquatic species that does not spend its entire life cycle in fresh water, or require fresh water to survive and reproduce.
Motile	An organism capable of active movement.
Passive surveillance	See 'general surveillance'.
Pathway	The geographic route taken by one or more vectors from point A to point B (see 'vector'). Pathways can be primary or secondary.
Pesticide	Any substance or preparation used for destroying a pest (typically associated with insects and rodents, with herbicides used for weed killers).
Plankton/planktonic	Small or microscopic organisms that drift or swim weakly in a body of water, including bacteria, diatoms, jellyfish, and various larvae (including bivalve larvae).
Primary invasion	Initial introduction of an introduced species in a disjunct region (e.g. located beyond a land, ocean, or temperature/salinity barrier). See 'primary pathway.'
Primary pathway	A primary pathway moves introduced species to new regions across biogeographic barriers (e.g. between continents).
Propagules	Dispersal agents of organisms, including spores, zygotes, cysts, seeds, larvae, eggs, sperm, and self-regenerative tissue fragments.
Regulation	A rule or order, as for conduct, prescribed by authority; a governing direction or law.
Route	A geographic track or corridor followed by one or more vectors (see 'vector' and 'pathway').

Term	Definition
Secondary invasion	Subsequent spread of an introduced species within a new region due to reproduction or translocation of the initial founder population. See 'secondary pathway.'
Secondary pathway	A secondary pathway is the spread and dispersal of introduced species between points within or between neighbouring regions (e.g. between local marinas).
Sedentary	An organism that may be capable of limited movement but typically remains in one place or moves little (e.g. infaunal bivalves). See also sessile.
Sessile	An organism that is immobile and typically attached to a surface or object for most or all of its life cycle.
Surveillance	Surveillance (also 'biosecurity surveillance') is the systematic investigation over time, of a population or area, to collect data and information about the presence, incidence, prevalence, or geographical extent of a pest or disease, and includes active and passive surveillance approaches.
Targeted surveillance	Surveillance which is undertaken to target marine pest species or taxa at certain locations and times. Targeted surveillance is usually done as part of active surveillance programs.
Translocate/translocation	Any deliberate or unintentional transfer of an organism or its propagules between disjunct sites.
Validation	A process that determines fitness-for-purpose of a specific test or assay. The validation process takes into account test sensitivity, specificity, repeatability, and robustness.
Vector	Vectors are the physical means, agent, or mechanism that facilitates the transfer and introduction of marine pests, or their propagules, from one place to another (e.g. vessels or maritime equipment).
Vessel	Any ship, boat, or other craft used in marine environments; includes ships, floating platforms, boats, and barges (e.g. structures that can float and be steered or moved by their own means or by other means, e.g. if towed). Also specifically includes smaller craft including recreational boats and other craft.

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