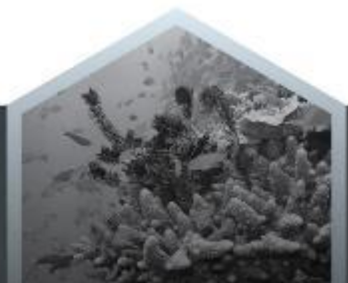
Australian Government

MARINE PESTS

Response manual for invasive ascidians

Version 1.0, March 2026

Nationally agreed guidance material endorsed by the Marine Pest Sectoral Committee



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Acknowledgement of Country

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

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 support 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 consistent with 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 response.

This manual describes practical management for a response to an incident caused by the suspicion or confirmation of incursion by an invasive ascidian.

Earth Sciences New Zealand, 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 1 September 2025.

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 (Cwth) *Biosecurity Act 2015* or aligning jurisdictional biosecurity legislation, 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, forward your suggestions to:

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

Version history

Version	Date	Amendment details
1	17 March 2026	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; Hilliard 2004; Wotton & Hewitt 2004).

Manual purpose

Emergency response operations are most effective if they are based on detailed knowledge of the life history, biology and ecology of the marine pest, its ability to introduce or carry pathogens, and susceptibility to control or eradication measures. 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 provide guidance and serve as a reference for managing emergency response and significant range extensions of invasive ascidians.

Emergency Marine Pest Plan Series

The Marine Pest Response Manuals are a series of guidance documents that provide information and guidance for 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
- Response manual for invasive ascidians
- Northern pacific seastar (*Asterias amurensis*)
- Japanese seaweed/Wakame (*Undaria pinnatifida*).

These response manuals offer guidance on the types of information needed to inform a 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 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 ascidians 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 ascidians 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 ascidian. 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 ascidian. The manual is 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 ascidian biology and provides a brief overview on the management of invasive ascidians.

There are six main sections within this manual and five appendices (Appendices A-E). The inclusion and exclusion of ascidian taxa covered throughout the manual and in Appendix A 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
- methods for detecting invasive ascidians using settlement arrays.

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 ascidians and how to respond to an incursion of any invasive ascidian, including species which may not be listed in this manual. Given the extraordinary diversity and complexity of ascidians, this manual does not focus on individual species. Instead, the scope of this manual is to consider ascidian diversity broadly, by either using functional groups such as solitary and colonial species, or by using broad taxonomic groups, such as the orders Aplousobranchia, Phlebobranchia, and Stolidobranchia. Throughout the manual, case studies involving particular species are used as examples for how technical information can be used to inform an incursion response.

Appendix A provides taxon-specific information for all ascidian species cited in this manual, including 11 high-priority families. This manual does not cover all invasive ascidians 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. Because new invasive species have the potential to be introduced to Australia via international pathways, overseas research on invasive ascidian species has relevance for incursion responses in Australia. Other taxa may be identified for inclusion or exclusion and the manual will be updated as required.

The invasion status of some ascidian species in Australia is currently limited due to the difficulty of detecting and identifying ascidians. *Didemnum vexillum* is currently listed on the Australian Exotic Environmental Pest List (EEPL). Considerations are underway as part of a review of the EEPL to determine triggers and processes to remove species when they are no longer considered 'exotic', including for *D. vexillum*. Although they did not meet the criteria for inclusion, several ascidians were considered against the Australian Priority Marine Pest List (APMPL); information on these species can be found in the APMPL Process and outcomes report. Known occurrences of such taxa in Australia is recorded in research literature and in online databases such as NIMPIS, World Register of Marine Species (WoRMS), and in the Smithsonian Environmental Research Center's National Estuarine and Marine Exotic Species Information System (NEMESIS) register.

General ascidian biology

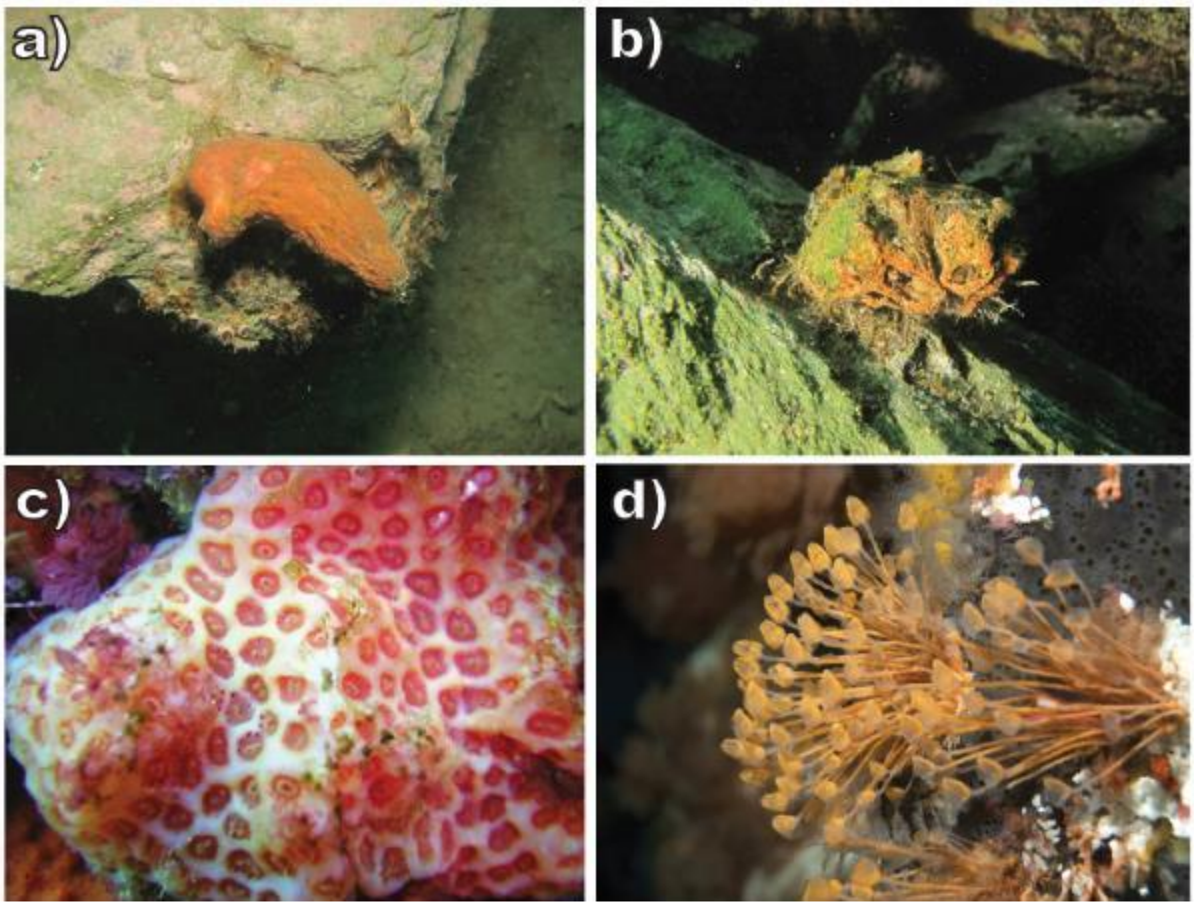
Taxonomy

Ascidians, commonly known as sea squirts, are invertebrate chordates (phylum Chordata, subphylum Tunicata, class Ascidiacea), a type of marine animal named for having an outer test or 'tunic coat' that can vary from a tough leathery flask in solitary species to a gelatinous colony of many small zooids (

Table **1**). The class Ascidiacea is represented by three orders: Aplousobranchia (e.g. *Didemnum*, *Aplidium*, *Polyclinum*, *Eudistoma*), Phlebobranchia (e.g. *Ciona*, *Ascidia*, *Corella, Perophora*) and Stolidobranchia, (e.g. *Pyura*, *Styela*, *Cnemidocarpa*, *Botrylloides*). Currently there are 25 families in the three orders; Aplousobranchia has 13 families, Phlebobranchia has 9 families and Stolidobranchia has 3 families (Shenkar et al. 2024).

There are currently 2990 described species of ascidians globally (WoRMS 2024), with an estimated 3000 species to still be discovered and described (Shenker & Swalla 2011). Expert taxonomic and/or genetic analysis for ascidian identification is often required due to the morphological similarity between invasive ascidians, and some native species. Ascidians can look like a simple warty lump, a massive cushion containing many clones or zooids, or appear as 'florets' of zooids joined at a basal mat (Photo 1). They inhabit marine and estuarine waters, can settle on natural and artificial substrate, and can generally tolerate a broad range of environmental conditions, making them capable invaders. No freshwater ascidians are known to exist.

Photo 1 Ascidian morphotypes



Photographs of ascidian morphotypes. 'Warty lump' solitary ascidians: a) *Cnemidocarpa bicornuta* from Doubtful Sound, Fiordland and b) *Pyura cancellata* from Waikawa breakwater, Picton, New Zealand; c) a massive cushion-like colonial ascidian *Aplidium* sp. from Three Kings Islands, New Zealand; d) *Pycnoclavella kottae* from the Three Kings Islands in New Zealand, showing 'florets' of zooids joined by a common basal mat. Sources: a – c) M Page, Earth Sciences New Zealand; d) M. Francis, Earth Sciences New Zealand.

Ascidian terminology used through this manual can be found in

Table **1**. They have been provided here for the comparison with photos and figures in the Introduction.

Table 1 Ascidian terminology for comparison with photos and figures in the Introduction

Term	Definition
Atrium	The cavity around the sides of the branchial sac, it separates the branchial sac from the body wall.
Atrial siphon	Is located at the exhalant aperture on the dorsal end of the ascidian. Water filtered in the branchial sac is passed out of the animal via the atrial siphon. It is often directed away from the animal down current to expel faeces and liberate gametes or larvae.
Basal mat	Anterior end of the test attached to the substrate, it can form attachment roots or hairs in solitary species or in colonial species, be a gelatinous mat in which zooid abdomina are embedded.
Branchial sac (feeding basket/pharynx)	Water in the atrial cavity is drawn in through the branchial siphon by cilia beating in the branchial sac.
Branchial siphon (oral/buccal siphon)	The inhalant siphon, where waters is drawn into the branchial sac.
Bud (blastozooid)	Blastozooids are asexual buds formed by precocious budding of an oozooid or larva (a fertilized egg) in the Didemnidae, and from asexual budding of zooids in other colonial taxa.
Canal	Common cloacal canals are aquiferous systems in colonial ascidians connecting zooid atrial apertures to exhale water and gametes to the surface of the surface of the colony via common cloacal apertures.
Colonial	Zooids connected or imbedded in a common test or basal mat.
Endostyle	A grove that runs the mid-line of the branchial sac lined with cilia which secretes mucus that is moved over the branchial sac to capture food.
Fragmentation	Dislodgement of a fragment of a colonial ascidian that is capable of dispersal, settlement and asexual reproduction in a new location.
Exhalant and inhalant apertures	Common to all ascidians – water needs to circulate to feed the animal. Individual siphons circulate water for solitary species. In colonial species (i.e. social or compound ascidians), inhalant apertures are normally separate with exhalant apertures opening below the surface of the colony to common canals, which then open to the surface through larger common cloacal apertures. The large cloacal apertures of colonies are often immediately visible.
Pharyngeal slits (stigmata)	Ciliated slits in the branchial sac, their morphology is used as a distinguishing character in orders Phlebobranchia and Stolidobranchia.
Propagule	Larvae or fragments that give rise to a new individual organism.
Spherule	An asexual bud.
Stolon	Stolonic vessel is an epidermal extension of the body wall in colonial zooids, homologous with the test vessel in solitary forms, these form the vascular system returning blood to the zooid test.

Test (tunic)	The often tough and leathery outer layer protecting solitary species or a firm or gelatinous matrix embedding zooids in colonial species. It is made of a polysaccharide tunicin, often coated with sand to form a hard protective layer. Blood or test vessels branch in the test to terminate in rounded ampullae near the surface.
Texture	For colonial species, this character can be important in distinguishing various taxa. The test can be gelatinous to the touch; this character is not common to sponges. Solitary ascidians have a test that can be leathery, cartilaginous, or thin and transparent. Colonial *Didemnum* spp. have spicules that are minute skeletal structures embedded in, or on the surface. These structures can make colonies brittle or feel like sandpaper. Sand grains can also be incorporated into tests. However, if the colony is cut or torn, the generally gelatinous test can be observed between zooids. The remaining body of the animal can be removed from the test (see Figure 1).
Visceral cavity	Peribranchial cavity, the space between the body wall and the viscera.
Zooid	A zooid is a single element of a colonial ascidian, analogous to an individual. An oozooid is a zooid formed via sexual reproduction from an egg (oocyte), whereas a blastozooid is a zooid formed via asexual budding (see 'bud' above).

Forms

Ascidians can be categorised into three forms: simple, social, or compound (Aldred & Clare 2014; Ayre et al. 1997; Brunetti & Mastrototaro 2004). Simple ascidians are usually referred to as solitary ascidians, meanwhile social and compound ascidians are often collectively referred to as colonial ascidians.

Solitary ascidians

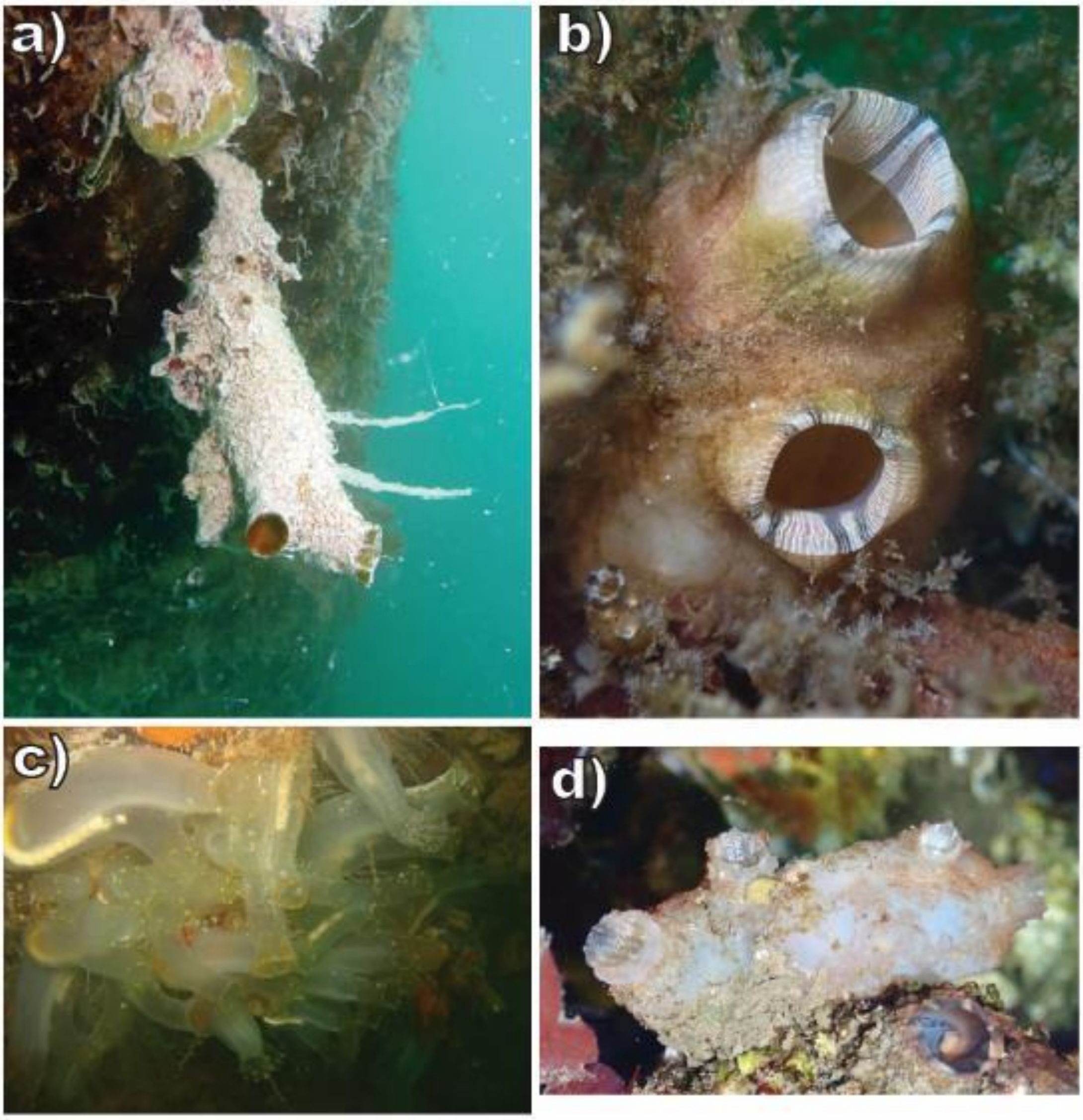
Solitary ascidians consist of a single discrete individual with its body attached to a substrate by its base or a stalk and encased in a test (or tunic) which is usually tough and leathery, especially in large specimens (Phlebobranchia, Stolidobranchia; Photo 2). Solitary ascidians may occur as abutting clumps, but the key feature is that each has originated from a separate larva. Social ascidians

Social ascidians consist of a group of individuals or zooids ('individual' replicating units), which have arisen through asexual budding from a single larva and are connected by a thin basal mat or a stolon (a social version of a test) (Photo 2d). Social ascidians are thus clones and grow together to form a colony. The test is usually thin and delicate and may be encrusted lightly with sand. Individuals are usually less than a centimetre or two in the longest dimension, but colonies may occupy several square metres on rocky vertical subtidal walls. In social ascidians the zooids are larger in size (>10 mm) than in compound species, and their inhalant apertures are visible on each individual zooid.

Compound ascidians

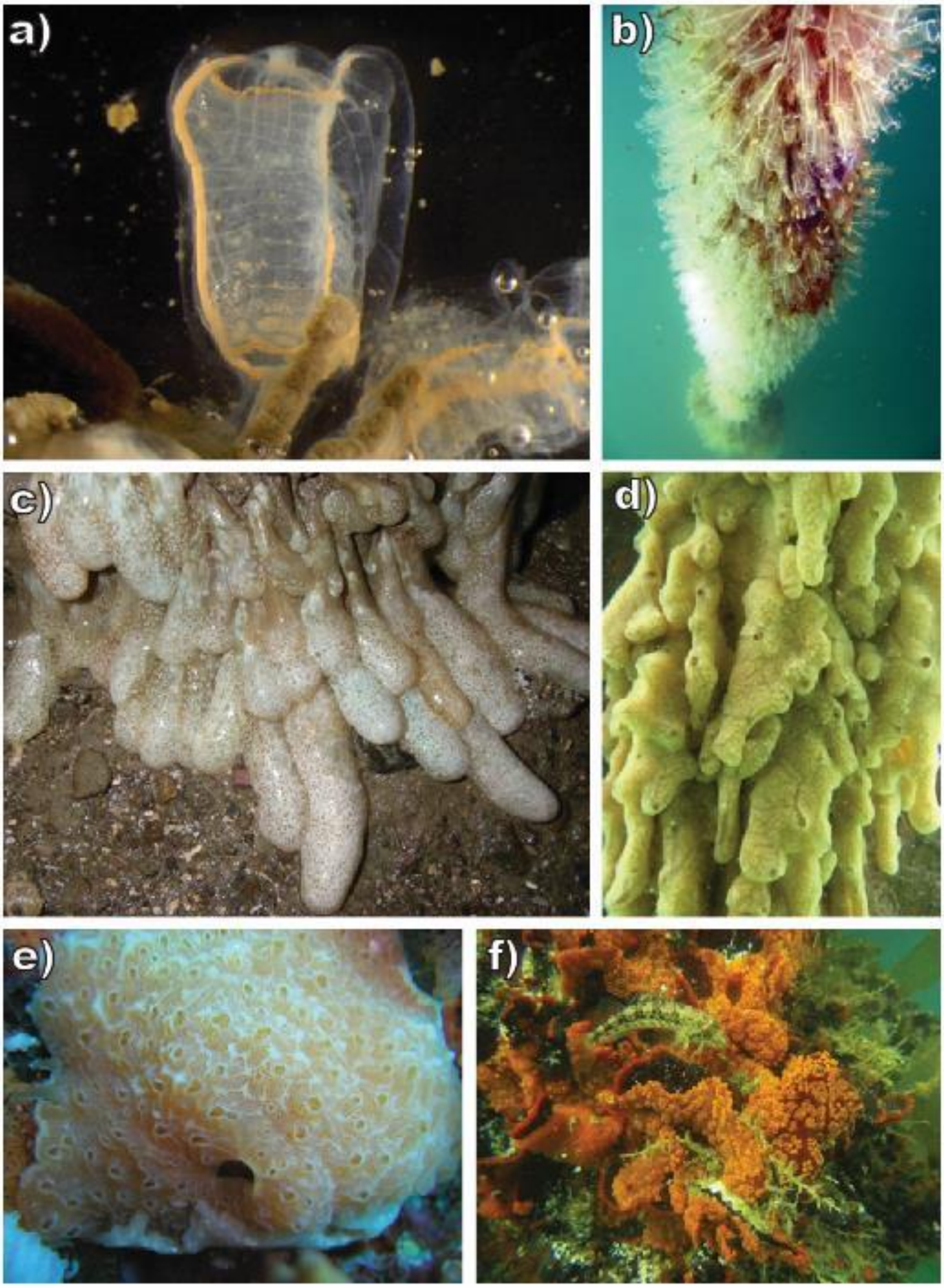
Compound ascidians are a group of many small (as small as 2 mm) zooids which have arisen through asexual budding from a single larva, and which are encased in a common matrix or test. The zooids may or may not be visible from the exterior of the colony and are generally less than a centimetre long (Photo 3a, b). Colonies of compound ascidian species occur as gelatinous jubes (Photo 3c), thin leathery sheets, small knobs, or cushions, or sprawling fleshy masses (Photo 3d-f). Many species are heavily impregnated and encrusted with sand, a characteristic that may make them difficult to initially identify as ascidians. Usually, obvious exhalant apertures with many small inhalant apertures are arranged along canals below the surface of the colony. They are often confused with sponges.

Photo 2 Examples of solitary ascidians



a) *Styela clava* from Picton, New Zealand; b) *Styela plicata* from Mission Bay, California, USA; c) *Ciona intestinalis* and *Ciona savignyi* from Whangārei harbour, New Zealand; d) *Ascidiella aspersa* from Vissschershoek, Netherlands. Sources: a, c) C. Woods, Earth Sciences New Zealand; b) Madeleine Claire; d) Jean-Paul Boerekamps.

Photo 3 Examples of colonial ascidians



a) *Clavelina lepadiformis* zooid from the Marlborough Sounds, New Zealand; b) a *Clavelina lepadiformis* colony encrusting a mooring line from the Marlborough Sounds, New Zealand; c) *Eudistoma elongatum* colonies in Whangārei Harbour, New Zealand, with zooid apertures opening externally without forming common cloacal systems; d) *Didemnum vexillum* at Shakespeare Bay, Picton, New Zealand, with zooids arranged around radial branching common cloacal canals opening to large common cloacal apertures on lobes; e) *Aplidium* sp. from the Marlborough Sounds, New Zealand, showing obvious double rows of inhalant zooid apertures arranged along common cloacal canals that coalesce at common cloacal apertures; f) *Botrylloides diegensis* from , with pigmentation around branchial apertures common to many botryllid species. *Sources:* a – f), M. Page, Earth Sciences New Zealand; b) C. Woods, Earth Sciences New Zealand.

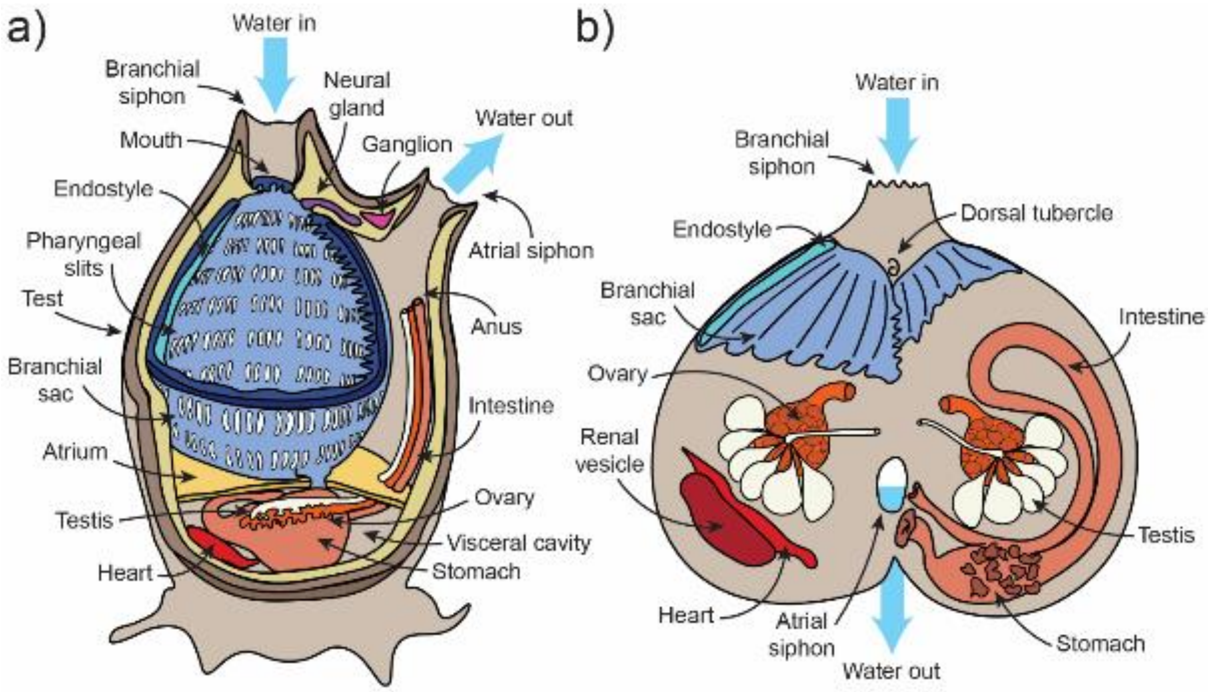
Error! Reference source not found. Table 1 provides descriptions of traits that may be used for i dentifying ascidians in the field without detailed taxonomic knowledge, although some terminological knowledge is required.

Anatomy

The zooid represents either the individual in solitary ascidians, or the base replicating unit in colonial forms. Ascidian zooids can be large (over 1 metre in length in the stalked *Pyura* species) or up to 30 cm high in Antarctic species, such as *Cnemidocarpa verucosa* (McClintock, 1991). They can also be very small, less than 2 mm in length as is common in colonial didemnid species. The zooid however has a standard structure (Figure 1). The zooid consists of a test (or tunic), lined by a mantle, that encloses a feeding basket – the branchial sac – and a gut loop. Water enters an ascidian through the branchial siphon, and it passes through the branchial sac via beating cilia that line the branchial sac and pharyngeal slits. Filtered water is then passed out through the atrial siphon. Food is trapped on a mucous net strung around the stigmata and pulled into a cord along the endostyle which runs down the branchial sac and then then enters the gut. The gut is a simple loop with an oesophagus, stomach, and small intestine. See

Table **1** for a glossary of ascidian terminology and Figure 1 for an illustration of the internal anatomy of solitary ascidian.

Figure 1 Internal anatomy of a solitary ascidian



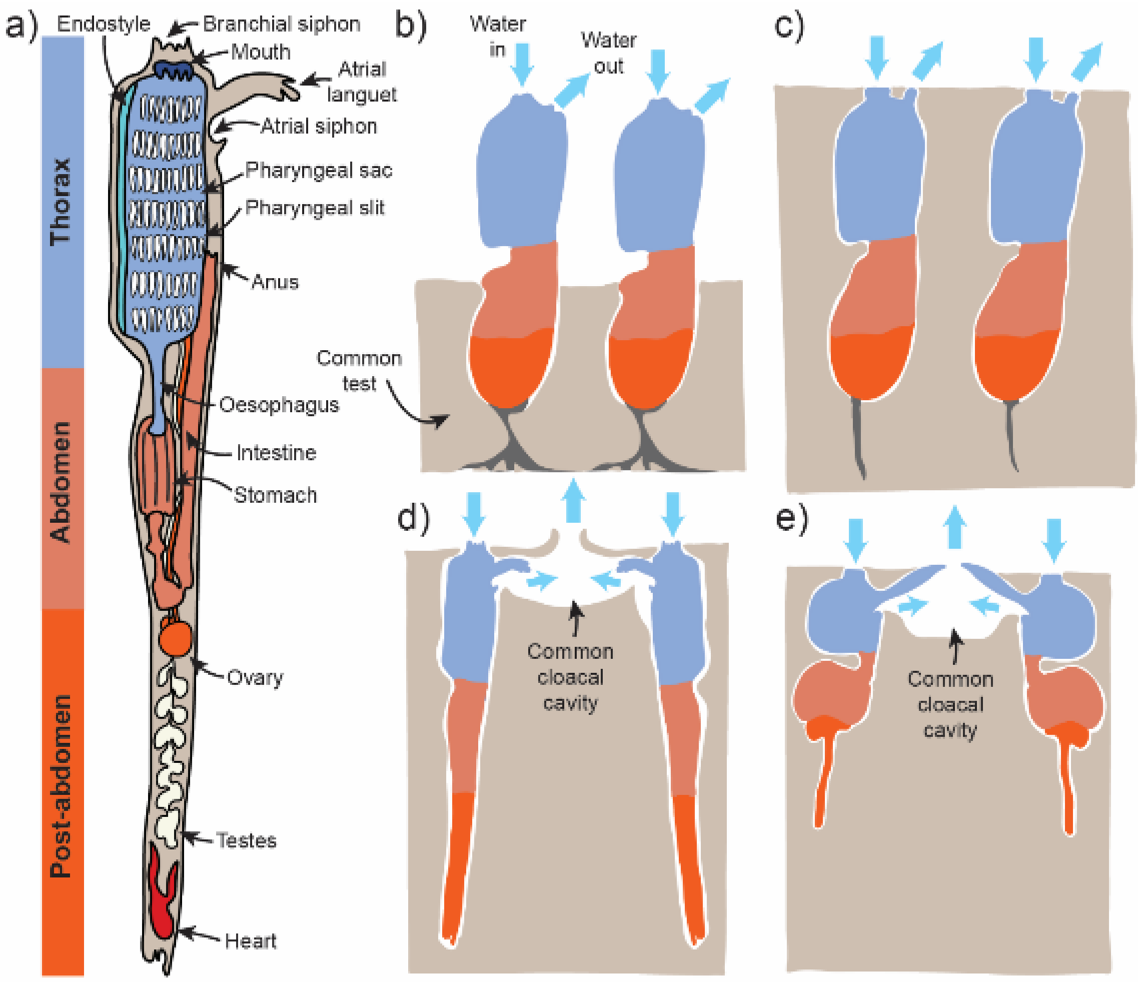
Two illustrated diagrams showing the internal anatomy of a solitary ascidian: a) a generalised solitary ascidian body plan; b) a diagram of a stolidobranch solitary ascidian, opened along the ventral line with the test removed. The branchial sac is partially removed to show gonads and digestive organs on the body wall (adapted from Monniot, C, Monniot, F & Laboute, P 1991 'Coral Reef Ascidians of New Caledonia'. Marseille, IRD Éditions, 145p). In both diagrams, arrows indicate the flow of water through the branchial siphon and sac and out through the stomach, intestine, and atrial siphon.

Ascidians have a simple nervous and circulatory system with a heart that reverses blood flow from time to time. Reproductive organs are located near the gut loop and their relative disposition to the gut is the basis for classification of orders:

- Aplousobranchia have a gut loop posterior to the branchial sac, the body is divided into thorax abdomen and sometimes post-abdomen, and are generally colonial species (e.g. *Didemnum*, *Aplidium*, *Polyclinum*, *Eudistoma*)
- Phlebobranchia gonads are usually on the same side of the body as the gut loop, never on both sides and the branchial sac is never folded (e.g. *Ciona*, *Ascidia*, *Corella, Perophora*)
- Stolidobranchia gonads are usually on both sides of the body and the branchial sac is usually folded (e.g. *Pyura*, *Styela*, *Cnemidocarpa*, *Polyzoa, Botrylloides*).

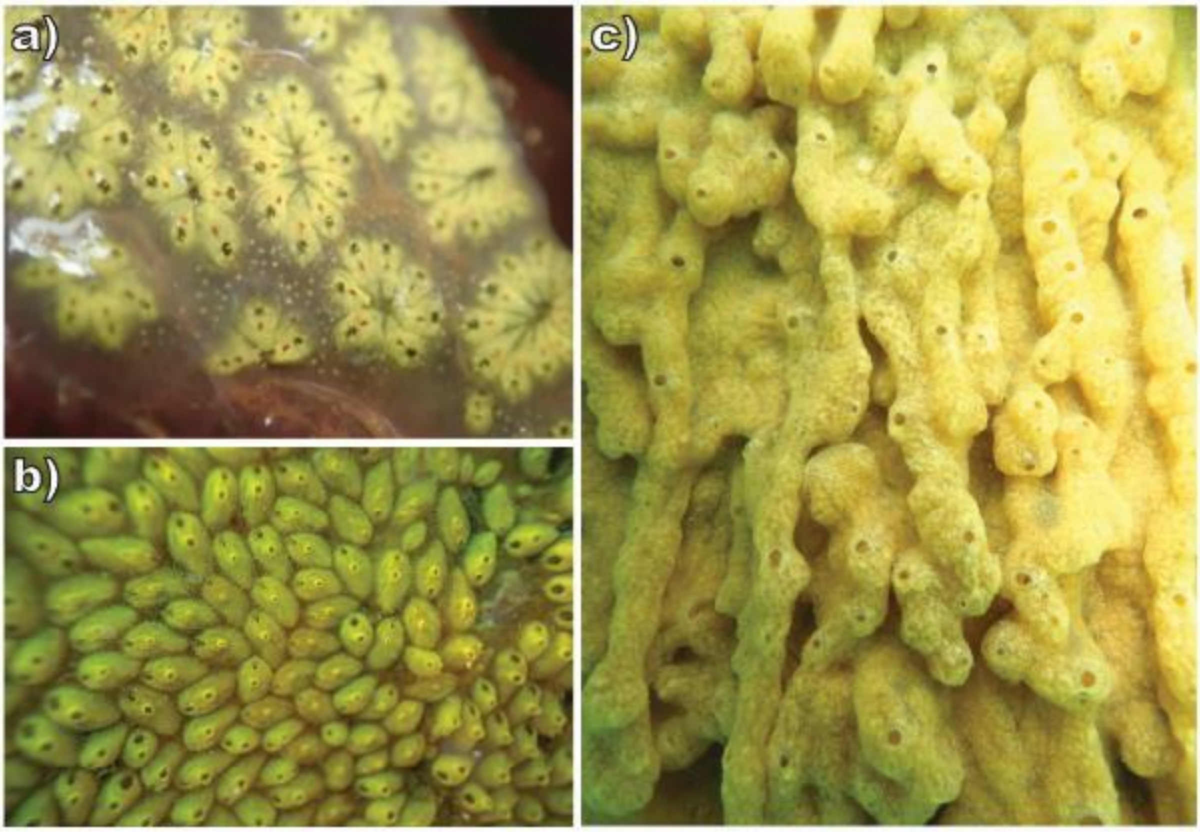
In colonial forms, atrial apertures of zooids embedded in a common test may be connected by a common cloacal canal below the surface of the colony that reticulate to a common cloacal (exhalant) aperture. Zooids within colonial species connected by this common canal share a vascular system (Brunetti & Mastrototaro 2004; Grosberg 1988; Manni et al. 2007; Mukai et al. 1987; Wawrzyniak et al. 2021) (Figure 2; Photo 4). The arrangement of systems and the disposition of the gonads are some of the characters used to discriminate species at the family level. Stolidobranch ascidians in the genera *Botryllus, Botrylloides* and *Symplegma* spp. also have zooids that form colonies with systems (Figure 3).

Figure 2 The internal anatomy of colonial ascidians



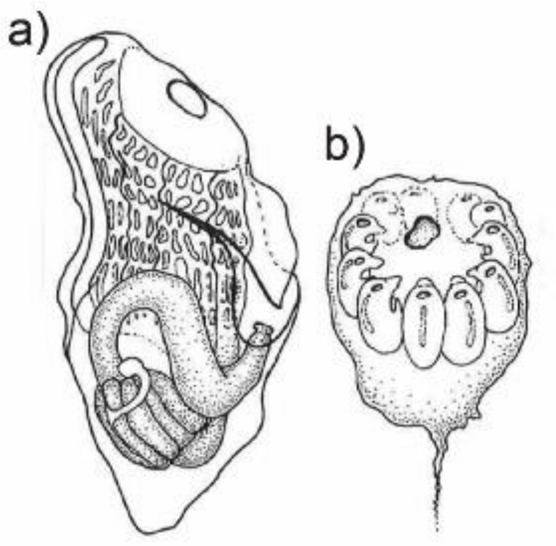
Five illustrated diagrams showing the internal anatomy and organisation of colonial (polyclinid) ascidia zooids. a) the internal anatomy of a colonial ascidian zooid, showing the division of the body into thorax, abdomen, and post-abdomen. Illustrations showing zooid organisations: b) partially embedded zooids (*Clavelina*); c) completely embedded zooids forming colonial, but not cloacal systems (*Eudistoma* spp.); d) zooids embedded with gonads in a post-abdomen with a common cloacal cavity (*Aplidium*, *Synoicum*); e) zooids formed into well-developed cloacal systems and wide extensive cloacal cavities with branching systems opening to large common cloacal apertures with gonads contained in the gut loop (*Didemnum, Lissoclinum*). Sources*:* a) is adapted with permission from (adapted from Monniot, C, Monniot, F & Laboute, P 1991 'Coral Reef Ascidians of New Caledonia'. Marseille, IRD Éditions, 150p.; a – d) adapted from (Kott 2003) with permission from Queensland Museum.

Photo 4 Example organisations of colonial ascidians



Close-up photos showing different organisations among various colonial ascidian species. a) *B. schlosseri* (compound) from Falmouth, UK; b) *S. brakenhielmi* (social) near Rakino Island, New Zealand; c) *D. vexillum* (compound) from Shakespeare Bay, Picton, New Zealand.. *Sources:* a) Ben Holt; b) Shaun Lee; c) C. Woods, Earth Sciences New Zealand.

Figure 3 Diagram of a colonial ascidian zooid



Two illustrations showing zooids of the colonial ascidian *Botrylloides saccus*; a) a zooid; b) a colony lobe showing circular orientation of zooids around a common cloacal aperture. Figure reproduced from Kott (2003) with permission from Queensland Museum.

Reproduction, life cycle, and growth

Many ascidian species are capable invaders due to their potentially rapid reproduction and growth. Most ascidians are hermaphrodites, and while many species are self-sterile to avoid inbreeding, this condition means that very few individuals are required to establish a population. Natural dispersal by larvae is limited in most ascidians due to their short larval phase, but some species may also disperse via fragmentation or asexual budding.

Reproduction

While solitary ascidians are restricted to sexual reproduction (Alié et al. 2021), colonial species are capable of both sexual reproduction and asexual reproduction via budding and fragmentation (Figure 4) (Aldred & Clare 2014; Fletcher et al. 2013c; Manni et al. 2007; Muñoz et al. 2015).

Ascidians have evolved a variety of strategies to promote out-crossing and limit self-fertilisation. In the solitary genus *Ciona*, self-sperm are either less competitive than non-self-sperm or individuals are entirely self-sterile (Jiang & Smith 2005; Sawada et al. 2014). Individuals of *Styela* are typically self-sterile (Forrest 2013; Wong et al. 2011), although physical disturbance may cause male and female gonads to spawn simultaneously (Forrest 2013). In the colonial genus' *Botryllus* and *Didemnum*, species are sequential hermaphrodites and the peaks for egg and sperm production are offset in time to limit self-fertilisation (Gasparini et al. 2015; Stefaniak & Whitlatch 2014).

Solitary ascidians are typically broadcast spawners where sperm and eggs are released into the water column and fertilisation occurs externally (Aldred & Clare 2014; Bourque et al. 2007; Forrest 2013). The developed larvae are usually mobile, relying on stored egg yolk for nutrition (Aldred & Clare 2014; Lambert 2002). Some solitary ascidians produce mucus strings or foams to aggregate and aid the dispersal of gametes and larvae (Aldred & Clare 2014; Petersen & Svane 1995). Many colonial ascidians brood larvae, either in the atrial cavity in Polyclinidae (e.g. *Aplidium* spp.) or in the test (e.g. *Didemnum* spp. (Figure 4) (Daley & Scavia 2008; Lambert 2002, 2009; Mastrototaro et al. 2019; Mukai et al. 1987; Muñoz et al. 2015; Ordóñez et al. 2015; Stefaniak & Whitlatch 2014).

Life cycle

Like all chordates, ascidians possess a notochord, paired lateral slits in the wall of the pharynx and a dorsal hollow nerve chord (Aldred & Clare 2014). In ascidians these three features co-occur only during the larval phase. Once the larva has settled, the animal takes up a sessile habit. During the adult phase, only the pharyngeal slits remain to indicate the chordate origin. Most species have indirect development, with a 'tadpole' like larval phase, although a minority of species exhibit direct development (Bates 1995). The body plan and overall morphology of the ascidian has a great variety of forms.

In most ascidians, embryogenesis is rapid (Manni et al. 2007); larvae settle within minutes or hours (rarely >24 hours) and generally do not travel any further than several kilometres (Aldred & Clare 2014; Atalah et al. 2021; Bourque et al. 2007; Fletcher et al. 2013c; Forrest 2013; Lambert 2002; Page et al. 2011; Petersen & Svane 1995; Simkanin et al. 2016; Stefaniak & Whitlatch 2014). See Section 2.4 below for a detailed discussion of natural dispersal ability. Larvae are negatively phototactic prior to settlement, leading to the colonisation of shaded locations such as docks, pontoons, and vessel hulls (Aldred & Clare 2014; Daley & Scavia 2008; Deibel et al. 2014).

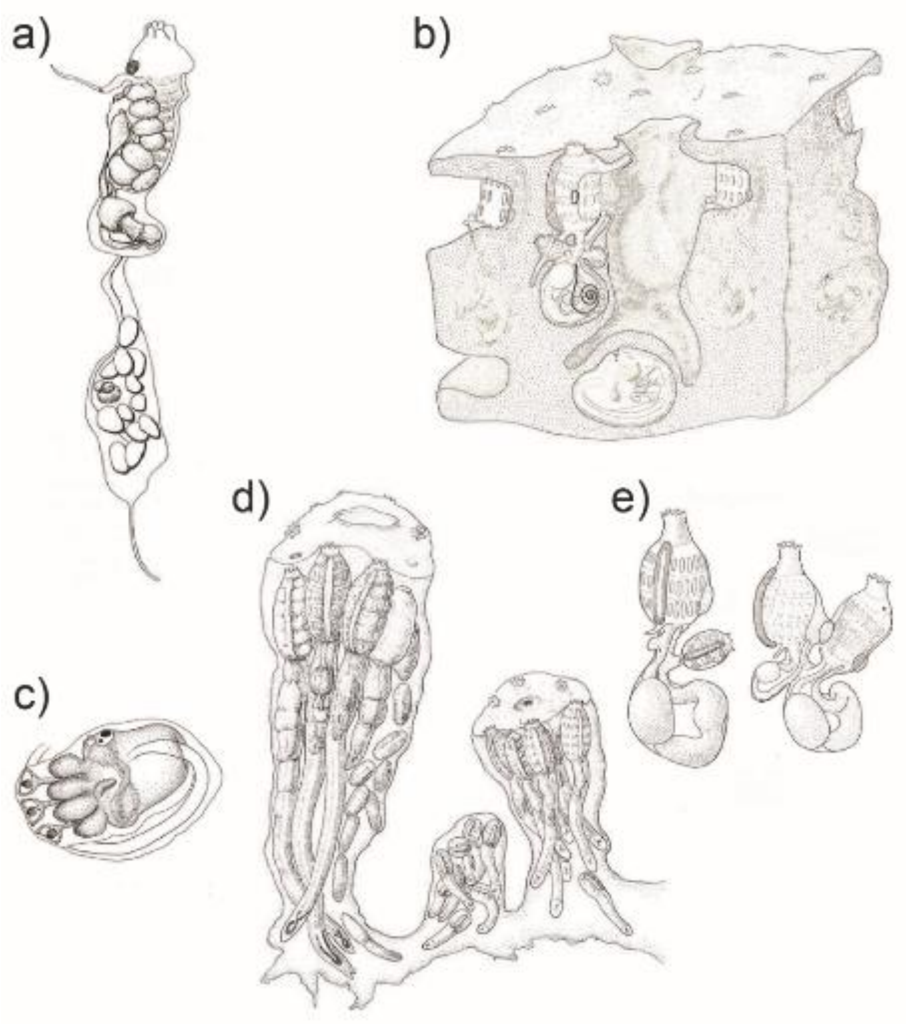
Both solitary and colonial ascidians can reach sexual maturity within a matter of weeks (Daley & Scavia 2008; Forrest 2013; Stefaniak & Whitlatch 2014; Zhan et al. 2015). Generally, all sexually mature zooids within colonial species develop a testis and an ovary on opposite sides of the body (Mukai et al. 1987), and the reproductive cycle is synchronised among zooids (Grosberg 1988).

Ascidians exhibit many different life history strategies, and the number of lifetime reproductive efforts can vary among individuals of the same species (Grosberg 1988). Many invasive ascidian species have long reproductive seasons, covering eight months or more (Deibel et al. 2014; Wong et al. 2011). The reproductive season of *D. vexillum* in New Zealand extends for up to 9 months (Fletcher et al. 2013a). Growth and reproduction in species are often correlated with temperature. In *D. perlucidum* and *D. vexillum*, reproduction and growth typically peak in warm summer months (Fletcher et al. 2013a; Muñoz et al. 2015), whereas other colonial ascidians concentrate activity in autumn and winter (Deibel et al. 2014; Muñoz et al. 2015).

Growth and asexual reproduction

Growth of social and compound ascidians is by continual budding (blastogenesis) of zooids (Figure 4) (Blanchoud et al. 2018; Grosberg 1988; Gutierrez & Brown 2017; Kassmer et al. 2019; Nourizadeh et al. 2021; Ricci et al. 2022; Scelzo et al. 2019). Budding can occur in several ways, either from the base of the colony, stolonal (e.g. *Perophora*); from elaboration of constricted parts of the entire zooid, strobilation (e.g. *Polyclinum*); or from budding at the abdomen/thorax juncture leading to two new zooids, one with an old thorax and a new abdomen and the other with an old abdomen and a new thorax, oesophageal-rectal budding (e.g. *Didemnum*). During cooler winter temperatures, many colonial species can survive through dormant, asexual buds (sometimes referred to as spherules) that are often more resistant to environmental stressors (Deibel et al. 2014; Page et al. 2011; Tobias-Santos et al. 2024). In colonial species, asexual growth via budding usually decreases while sexual reproduction peaks (Deibel et al. 2014).

Figure 4 Brooding and asexual growth in colonial ascidians.



Examples of variation in brooding and asexual growth in colonial ascidians; a) polyclinid larvae brooded in a zooid peribranchial cavity adjacent to the branchial sac, to be released through the atrial aperture on maturation; b) didemnid larvae brooded in the ventral test below the common cloacal canals, to be released as a developed tadpole from a common cloacal aperture; c) a larva of *Didemnum roberti* shows 3 adhesive papillae used by the larva for attachment to the substratum. The sensory vesicle is a black structure, it contains a statocyst for balance and a light-sensitive ocellus; d) an example of polyclinid strobilation forming a colony; e) oesophageal-rectal budding in the Didemnidae, a thoracic bud differentiates from the oesophagus while a rudimentary abdominal bud develops, these then join to the old abdomen and the old thorax, respectively. The zooids then split-off forming two individuals. Source: Figures a, c, reproduced from Kott (1992) and Kott (2001) with permission from the Queensland Museum; figures b, d, e) © IRD / F. Monniot, reproduced with permission from Monniot, C, Monniot, F & Laboute, P 1991 'Coral Reef Ascidians of New Caledonia'. Marseille, IRD Éditions, 248 p.

Fragments of colonial ascidians can break off and surviving buds can establish new colonies, and some species grow into tendril-like shapes to encourage this process (Bullard et al. 2007; Coutts & Forrest 2007; Lambert 2002; Nishikawa et al. 2000; Stefaniak & Whitlatch 2014; Turon & Becerro 1992). Some genera, such as *Botrylloides*, *Botryllus*, *Symplegma*, and *Polyandrocarp*a, are also capable of whole-body regeneration where circulating stem cells allow individuals to regrow following damage, even from fragments of vascular tissue (Blanchoud et al. 2018; Brown et al. 2009; Gutierrez & Brown 2017; Kassmer et al. 2019; Nourizadeh et al. 2021; Ricci et al. 2022; Scelzo et al. 2019; Wawrzyniak et al. 2021). In such cases, asexual reproduction is triggered by environmental interactions causing damage, whereas normal budding is triggered by the ascidian itself (Alié et al. 2021). Similarly, while not used for reproduction, some solitary ascidians such as *Ciona* can regenerate the oral siphon and their nervous system from stem cells stored in the branchial sac (Kassmer et al. 2019).

Ascidian ecology

Feeding and competition

Ascidians are suspension feeders largely feeding on micro algae and bacteria, pumping water in from the water column through an inhalant aperture (branchial siphon). Food particles captured are moved by a mucus net excreted from the endostyle and moved over the branchial sac by cilia. Up to 100% of particles of 1–2 µm in size and particles as small as bacteria can be retained by ascidians (Petersen 2007). While competition for space and physical smothering of other species is the main impact of biofouling ascidians, species such as *Styela clava* and *Ciona* spp. also appear to compete for food with cultured shellfish such as mussels (McKindsey et al. 2009) and scallops (Uribe & Etchepare 2002). Ascidians may have a competitive advantage in waters with low food concentrations dominated by small phytoplankton and bacteria (Petersen 2007). Clearance (feeding) rate in ascidians is correlated to water temperature (Ribes et al. 1998), food particle concentration (Hoxha et al. 2018), capacity of the digestive system and enzyme kinetics (Petersen 2007).

Predators

Little is known about predation upon ascidians, despite the abundance and widespread distribution of some invasive ascidians (Bullard et al. 2007; Epelbaum et al. 2009). Most predation appears to occur during larval stages or shortly after settlement (Muñoz & McDonald 2014; Osman & Whitlatch 2004; Stefaniak 2017). Larval predators include jellyfish and anemones (Atalah et al. 2013; Petersen & Svane 1995). Adult ascidians and colonies face a variety of benthic grazing predators, including sea urchins and sea stars (Atalah et al. 2014; Bullard et al. 2007; Epelbaum et al. 2009; Fletcher 2014; Petersen & Svane 1995), shrimp (Dumont et al. 2009), crabs (Carver et al. 2003; Epelbaum et al. 2009), nudibranchs (Epelbaum et al. 2009), gastropod molluscs (Atalah et al. 2014; Epelbaum et al. 2009; Fletcher 2014; Osman & Whitlatch 2004; Simkanin et al. 2013), littorine snails (Bullard et al. 2007; Carman et al. 2009a; Valentine et al. 2007a), and demersal fish (Dijkstra et al. 2007; Fletcher 2014). Birds such as oystercatchers are also known to feed on some shallow water species (Fletcher 2014). Ascidians growing on the seafloor and suspended structures are also grazed by generalist feeding fishes (Epelbaum et al. 2009; Osman & Whitlatch 2004), although a lack of digestion in some species suggests that fish sometimes accidentally ingest ascidians while targeting other organisms (Valentine et al. 2007b). Species of ascidian that can grow on suspended structures are likely to benefit from reduced predation rates (Giachetti et al. 2020).

While ascidians are eaten by many of the species listed above, experimental trials indicate that they are not preferred prey (Epelbaum et al. 2009). Colonisation success of invasive species is dependent on predation pressure of early life stages. Juvenile ascidians are likely more vulnerable to predation than adults, however, chemical, and physical defences influence species-specific variability in predation (Giachetti et al. 2022). Didemnid ascidians produce chemical deterrents and exhibit a very low tunic surface pH, which reduces predation (Bullard et al. 2007). Vegetative growth in colonial ascidians generally seems to outpace predator consumption (Atalah et al. 2014). The ability of *D. vexillum* colonies to fuse and fragment, to colonise and disperse from suspended structures, to grow as mats on pebble substrates, and to embed calcified structures within their tunics also likely reduces the impact of predation (Stefaniak 2017).

Parasites and diseases

A variety of gregarine apicomplexan parasites are known to infect various ascidian species, including invasive taxa (Ciancio et al. 2001; Ciancio et al. 2008; Lynch et al. 2016; Mita et al. 2012; Rueckert & Leander 2008). In *Ciona intestinalis*, infections of *Lankesteria ascidiae* predominantly occur in the digestive tract, causing the stomach to turn pale, congestion in the digestive tube, and the excretion of long, thin faeces (referred to as 'long faeces syndrome' (Mita et al. 2012)).

Haplosporid chromist infections have also been reported in invasive ascidians such as *C. intestinalis* (Ciancio et al. 1999). The kinetoplastid protist parasite *Azumiobodo hoyamushi* is the causative agent of 'soft tunic syndrome' in wild and farmed *Halocynthia roretzi* (Hirose et al. 2012; Kim et al. 2014; Kumagai et al. 2011; Kumagai et al. 2010). The invasive ascidian *S. clava* is a potential asymptomatic carrier of *A. hoyamushi* (Kumagai et al. 2014).

Approximately 200 species of notodelphyid copepods are known to inhabit a broad diversity of ascidians, with most occurring in solitary taxa (Kim & Boxshall 2020). These copepod species inhabit the pharynx or atrium of their ascidian hosts. Most species are considered to be commensal, but some have been demonstrated to be parasitic (Illg 1970; Kim & Boxshall 2020; Tovar-Hernández et al. 2010). These copepods have been found in practically every ascidian genus with invasive species, including *Ascidiella, Botrylloides, Botryllus, Ciona, Didemnum, Diplosoma, Molgula, Polyandrocarpa, Pyura, Styela* and *Symplegma* (Dudley 1968; Hirose et al. 2004; Kim & Boxshall 2020; Marchenkov 1998; Saad 2016; Scippa et al. 2000).

Trematode and tubellarian platyhelminths are also known to infect invasive ascidians (Lynch et al. 2016).

It is plausible that invasive ascidians can act as reservoirs for parasites and viruses that can infect humans and a wide variety of marine organisms. Furthermore, invasive ascidians may introduce new diseases and parasites to native ascidians, harming local biodiversity (Lynch et al. 2016). The harmful shellfish parasite *Bonamia ostreae* (see Australia's National List of Reportable Diseases of Aquatic Animals) and the *Minchinia mercenariae*-like parasite have been confirmed by Sanger sequencing and histology in *S. clava* from Ireland (Costello et al. 2021). *Styela clava* can maintain viable *B. ostreae* in the absence of oysters under tank conditions, suggesting that ascidians can act as reservoirs of infection for *B. ostreae* (Costello et al. 2021). *Vibrio aestuarianus* has been confirmed by qPCR in *B. violaceus* and *D. vexillum* (Costello et al. 2021). Marine birnavirus (*Aquabirnavirus*) has been detected at high rates in the farmed ascidian *H. roretzi*, although infections were not associated with poor host condition or soft-tunic syndrome (Azumi et al. 2007). Similarly, cells of harmful algal bloom species have been demonstrated to survive digestion by invasive ascidian species, meaning that invasive ascidians can act as vectors for harmful algal blooms that may pose a threat to shellfish aquaculture (Rosa et al. 2013).

Environmental tolerances and habitat

Ascidians can generally tolerate a broad range of environmental conditions, making them capable invaders.

Most invasive ascidians can occur across a remarkably broad temperature range, generally between 0 and 25°C (Carver et al. 2006; Daley & Scavia 2008; Forrest 2013; Rocha et al. 2017; Valentine et al. 2007a). However, many species are also recorded from tropical waters reaching 34°C (Rocha et al. 2017). The most likely temperature range for *Didemnum vexillum* is 0–30°C (Summerson 2024). Reproductive activity of ascidians is generally limited by temperatures <12°C, although it is crucial to note that this does not mean a total cessation of reproduction in many invasive species (Fletcher et al. 2013a; Forrest 2013; Muñoz et al. 2015). In New Zealand, *D. vexillum* reproduction appears to not occur below 9°C (Fletcher et al. 2013a), and experiments indicate that individuals reach sexual maturity between 19 and 21°C (Stefaniak & Whitlatch 2014).

Short-term tolerance of temperatures outside their optimal thermal range may enable ascidians to survive transit through regions where they cannot establish permanent populations. This facilitates their spread to new areas.

Ascidians can generally tolerate salinities as low as 20 ppt (Dijkstra 2022; Forrest 2013) (see Section 5.3.2.3). Some stolidobranch ascidians such as *Styela* can temporarily withstand <24 hours exposure to very low salinities (≥8 ppt), as they can close their siphons for extended periods (Coutts & Forrest 2005; Forrest 2013).

Ascidians generally occur in shallow subtidal or deeper water, but some species can occur within the intertidal zone. Some solitary ascidians such as *Styela* spp. can also survive air exposure for days and weeks (Hillock & Costello 2013) (see Section 5.3.1.1). Since ascidian larvae are negatively phototaxic they avoid well-lit areas (Aldred & Clare 2014; Daley & Scavia 2008; Deibel et al. 2014), and both adult and juvenile ascidians are vulnerable to direct exposure to sunlight (Bingham & Reitzel 2000).

The asexual buds (spherules; see Reproduction, life cycle, and growth) produced by some colonial ascidians (Deibel et al. 2014; Page et al. 2011; Tobias-Santos et al. 2024) are likely to allow species such as *Polyandrocarpa zorritensis* to survive harsher environmental conditions outside of their usual tolerance ranges.

Distant populations of invasive taxa may also be locally adapted to different environmental conditions (Carver et al. 2006), which may positively or negatively affect survival rates during incursions. Increased ocean temperatures and ocean acidification are also likely to significantly expand suitable habitat and potential geographic ranges for many invasive ascidians (Lins et al. 2018; Peck et al. 2015; Zhang et al. 2020).

Table 2 summarises recorded temperature, salinity, and depth ranges for many invasive ascidian species.

Table 2 Environmental tolerances of ascidian species

Ascidian species	Temperature (°C)	Salinity (ppt)	Depth range (m)
Ascidiella aspersa	–0.3–30.6	18–40[c]	0–80[f]
Ascidia sydneiensis	1–34.7[b]	10–44[a]	Shallow subtidal
Botrylloides leachii (cf. jacksonianum)	3–28[a]	16–38[a]	0–100
Botrylloides giganteus	Warm temperate–tropical[j]	18–40[j]	Shallow subtidal
Botrylloides diegensis	18–35[j]	18–40[j]	Shallow subtidal
Botrylloides violaceus	5–34[a]	15–38[a]	Shallow subtidal
Botryllus schlosseri	3–29[a]	14–44[a]	Shallow subtidal
Ciona intestinalis	–0.6–0.5[a]	10–37[a]	1–500[i]
Ciona savignyi	0.3–31.1[b]	18–40[j]	1–500[i]
Ciona robusta	3.8–30.4[b]	5–45[a]	Shallow subtidal
Clavelina lepadiformis	1.1–29.1[b]	14[1]–40[e]	50[e]
Clavelina oblonga	1.2–31.4[b]	11–37[d]	Shallow subtidal
Diplosoma listerianum	9–39[a]	34–34[a]	Not reported
Didemnum perlucidum	15–31[f]	14–39.4[f]	1–8[f]
Didemnum vexillum	0–30[f]	10–33[f]	0–81[f]
Eudistoma elongatum	10–unknown[g]	20–unknown[g]	0–5
Microcosmus exasperatus	10.9–34.8[b]	12–32[a]	0–5
Microcosmus squamiger	10.3–33.7[b]	Not reported	0–35[h]
Molgula manhattensis	–1.5–32.1[b]	9–40[a]	0–5
Polyandrocarpa zorritensis	12–30[k]	22.7–38[k]	Shallow subtidal[l]
Pyura doppelgangera	Likely 12–unknown[m]	Not reported	0–12[m,n]
Styela clava	–2–24[f]	17.5–38.5[a]	0–25[f]
Styela plicata	2.5–32.5[b]	17.5–38.5[a]	0–30[f]
Symplegma brakenhielmi	15–22.9[o]	Not reported	0–30

[a]Rocha et al. (2017); [b]Lins et al. (2018); [c]Prakasam & Jayapaul (1990); [d]Majnarić et al. (2022); [e]Riley (2008); [f]NIMPIS; [g]Page et al. (2011); [h]Lowe (2002); [i]Carver et al. (2006); [j]NEMESIS; [k]Lambert & Lambert (1998); [l]Brunetti & Mastrototaro (2004); [m]Fletcher (2014); [n]Atalah et al. (2021); [o]Spyksma et al (2024).

Overview of invasive ascidian management

Management of invasive ascidians depends upon the target form (solitary or colonial) and species, as well as the size of an incursion (area infested and size of the target population) and location. The most appropriate management responses for ascidians use a combination of physical and chemical treatments (see Section 5). Where possible, hauling out affected surfaces and desiccation in sunlight, encapsulation and smothering are currently the best physical treatments, which can be complemented with effective chemical treatments such as acetic acid, quaternary ammonium compounds in detergents, or lime. To reduce the risk of further spread, care must be taken to avoid dislodging individuals or fragmenting colonial ascidians during the movement or encapsulation of fouled vessels and structures, as well as during any manual removal. During reproductive seasons (typically spring – autumn), managers should consider mitigation efforts to contain gametes and larvae and reduce larval settlement. Waste from decontamination efforts must be disposed of appropriately (see Section 6). Below we briefly summarise Australian and overseas biosecurity responses to some of these invasive ascidian taxa.

Didemnum perlucidum in Western Australia, Australia

In 2010, during routine introduced marine pest surveillance along the Western Australian coast, *D. perlucidum* was detected in the Swan River Estuary in Western Australia (Muñoz & McDonald 2014). It was subsequently detected at further locations across the state (Muñoz & McDonald 2014). Encapsulation using impermeable plastic wrapping complemented with acetic acid chemical treatment proved to be an effective control method for fouled structures (Muñoz & McDonald 2014). Where encapsulation was not viable in aquaculture settings, managers recommended manual removal combined with dipping or spraying with chemical treatments to eliminate *D. perlucidum* fouling on mussel lines (Muñoz & McDonald 2014). Hauling out and prolonged air-drying was recommended as an effective last-resort response, although such efforts were noted to be expensive and time consuming (Muñoz & McDonald 2014). Long-term eradication was deemed to be unlikely (Muñoz & McDonald 2014). *Didemnum perlucidum* is also established in parts of the Northern Territory since 2009 and Queensland (since 2020), and ongoing management is undertaken in these jurisdictions to ensure that these incursions do not spread further unchecked. The 'Potential distribution of the invasive marine species *Didemnum perlucidum* (white colonial sea squirt) in Australia' report prepared by the Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) shows that most of Australia, excluding the southern temperate coastline, may be susceptible to *D. perlucidum* establishment, hence the need for continued surveillance.

Didemnum vexillum in Western Australia and Australian territorial waters within New South Wales, Australia

In 2021, *D. vexillum* was detected early in its invasion at HMAS *Stirling*, Western Australia, during routine surveillance by the WA Department of Primary Industries and Regional Development (WA DPIRD). The incursion was delimited and subjected to control and eradication management activities, including diver assisted underwater vacuum, and vessel hygiene and inspection regimes by the Australian Government Department of Defence with advice from WA DPIRD. The ascidian's ability to recolonise cleaned infrastructure outpaced the underwater vacuum activities. In 2023, surveillance detected the spread of *D. vexillum* to Henderson, Western Australia, where a Quarantine Area Notice was put in place by WA DPIRD to mitigate the risk of spread by vessel movements.

In 2023, a detection of *D. vexillum* was made on a pylon of a wharf at Fleet Base East in Sydney Harbour, NSW. In 2024, the Consultative Committee on Introduced Marine Pest Emergencies (CCIMPE) recommended that it was not technically feasible to eradicate *D. vexillum* from these areas of detection in Australia. This recommendation was endorsed by the National Management Group (NMG) and the response transitioned to management.

Ongoing management activities have been designed to monitor the presence, spread, and extent of *D. vexillum* in Australia, and research and development into its biology and impacts has commenced. The Potential distribution of the invasive marine species *Didemnum vexillum* (carpet sea squirt) in

Australia report prepared by ABARES shows that southern Australia, from Shark Bay in WA through to the south of Gladstone in QLD, are potentially suitable for *D. vexillum* establishment.

Clavelina lepadiformis in South Australia, New South Wales and Victoria, Australia

In contrast to the other examples presented here, not all detected incursions of invasive ascidians have been deemed to warrant elimination efforts. In late 2021 and early 2022, *C. lepadiformis* populations were reported in various locations in South Australia, in Twofold Bay, New South Wales and Port Phillip Bay, Victoria. While colonies were abundant at these locations, *C. lepadiformis* was not behaving invasively and was restricted to disturbed habitat and fouling on artificial vertical structures. Given these observations, neither a formal response nor control activities were recommended, however, ongoing passive surveillance was supported to ensure that these incursions do not spread further unchecked.

Didemnum vexillum in the Marlborough Sounds, New Zealand

In 2001, *D. vexillum* was discovered on a barge in the Marlborough Sounds in New Zealand (Coutts & Forrest 2007). The subsequent response effort was ineffective for eliminating and containing *D. vexillum,* but the response effort provided significant learnings to improve future management efforts. The encapsulation of wharf piles using impermeable plastic wrapping was effective, as was seabed smothering on a gentle slope using uncontaminated dredge spoil (Coutts & Forrest 2007). Encapsulation was also effectively combined with chlorine chemical treatment (Coutts & Forrest 2007). However, smothering was ineffective over a steeper slope covered with rip-rap and the length of time between the detection and identification of *D. vexillum* was too long to prevent spread (Coutts & Forrest 2007). Fragments established satellite populations on the seafloor before smothering operations could be completed and biofouling emerged from the wrapped structures (Coutts & Forrest 2007). Effective pathway management and regular surveillance of vectors at high-risk sites and vessels are the key mechanisms to lower the probability of an incursion and to permit early detection before *D. vexillum* becomes established.

Eudistoma elongatum in Northland, New Zealand

In 2005, *E. elongatum* was detected in oyster farms in Northland, New Zealand (Morrisey et al. 2009; Page et al. 2011). Trials indicated that sprayed acetic acid was effective against colonies exposed at low tide, but other chemical and physical treatments such as heating with blowtorches were ineffective (Morrisey et al. 2009; Page et al. 2011). Effective management and surveillance of vectors in aquaculture (such as the movement of stock and equipment) was concluded to be crucial to prevent further spread of the species (Page et al. 2011).

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 their 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 ascidians 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 ascidians. Contact the MPSC Secretariat for more information: mpsc@aff.gov.au.

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

- The National Introduced Marine Pest Information System (NIMPIS)
- Marine Pest Response Manuals
- National Priority Pests: Part II Ranking of Australian Marine Pests
- 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

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 ascidian should engage as early as possible with the Consultative Committee on Introduced Marine Pest Emergencies (CCIMPE), ensuring ongoing collaboration throughout any response activity.

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 may be established to help manage concurrent incursions in more than one jurisdiction. National coordination operations will work in consultation with the 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 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 roles and responsibilities.

CCIMPE provides technical and response recommendations and advice to jurisdictions 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.

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 all of the following conditions are agreed to have been met: eradication is considered feasible, the pest is considered to be of national significance, and the benefits of a response outweigh the costs and 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 et al. (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 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 share 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 combat 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 ascidian not known to occur in Australia, or significant range expansions of established pests, 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. operational 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 ascidians 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 the current status of marine pests 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 current status of marine pests may influence management actions such as movement restrictions, ballast water and biofouling management. See Section 4 for more information on surveillance and delimitation.

The 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 current status (likelihood of presence/absence) of marine pests in defined areas within Australia.

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 current status of marine pests on request.

Ultimately, surveillance for current status of marine pests will depend upon the context and requirement. CCIMPE can provide advice and connection to expertise to assist in developing a surveillance plan to assess current status of marine pests 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.

[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. at a particular time. 'Proof of freedom' may still be used interchangeably in some circumstances or in older documents.

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. Operational staff should be appropriately trained to required WHS standards for all activities being undertaken during a response.

1.5.3 Environmental considerations

When a response takes place there may be associated waste or pollution 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 waste requires careful planning and appropriate disposal areas and transport corridors identified. When handling or destroying invasive ascidians, care must be taken to prevent induced spawning or breaking off asexual fragments, and all biological material collected and filtered out of effluent water to avoid reinfesting the marine environment. See Section 6 for more information on decontamination, destruction, and disposal.

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

Response actions also need to consider the surrounding environment. Some high priority areas such as marine reserves, Sea Country, national and marine 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 ascidians to and throughout Australia are provided in this section. Transport of biofouling on seagoing vessels and other maritime infrastructure is the vector considered to have the highest risk of introducing invasive ascidians to Australian waters.

Invasive ascidians can also be introduced and spread by:

- discharge of ballast water
- fisheries and aquaculture trade (via epibiosis)
- transport via recreational vessels and bilge water (Fletcher et al. 2017; Fletcher et al. 2021)
- natural dispersal (e.g. ocean currents)
- debris and flotsam.

The natural dispersal ability of ascidian larvae is limited, however, most ascidians can colonise a variety of surfaces, including jetties and vessel hulls, as well as the extremities of wild and farmed organisms, facilitating spread. Furthermore, most colonial species are capable of asexual reproduction, and fragmentation allows many invasive ascidians to quickly become established in new areas. The combination of multiple natural dispersal and human-mediated pathways can contribute to the spread of invasive ascidians .

Details of pathways and vectors for the introduction and spread of invasive ascidians 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.

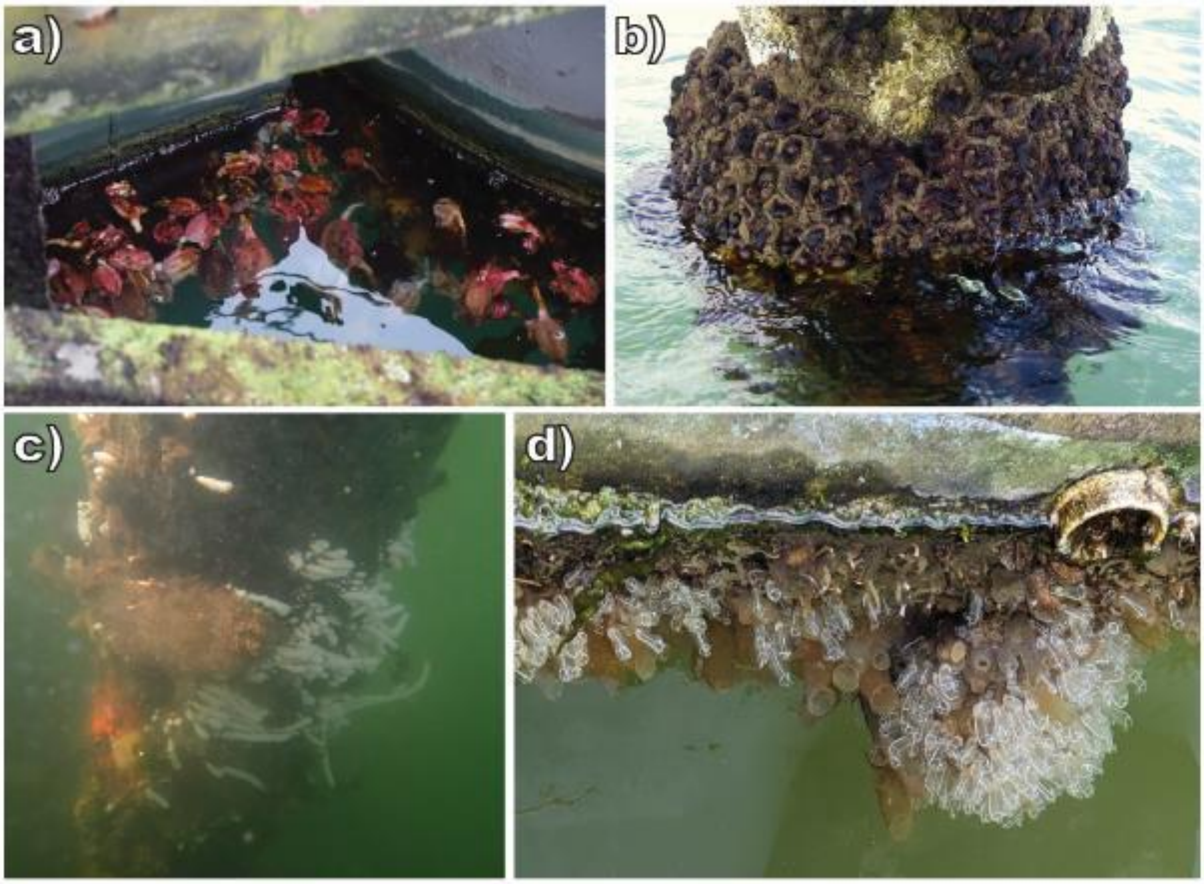
2.1 Biofouling

Risk of ascidian dispersal by biofouling

Ascidians are considered to be the most problematic of all fouling organisms (Aldred & Clare 2014), and they can colonise a wide variety of surfaces, including jetties, vessel hulls, sea chests, harbour walls, and other underwater niches (Bridgwood et al. 2014; Bullard et al. 2007; Coutts & Dodgshun 2007; Daley & Scavia 2008; Darbyson et al. 2009; Deibel et al. 2014; Forrest 2013; Lambert & Lambert 1998; Lambert 2002; Pleus et al. 2008; Simkanin et al. 2012) (Photo 5 and Photo 6). These shaded areas are preferentially colonised by ascidians because larvae exhibit negative phototaxis prior to settlement (Aldred & Clare 2014; Daley & Scavia 2008). Ascidians also frequently colonise aquaculture equipment such as nets, cages, lines, buoys and piles (Carman & Grunden 2010; Carver et al. 2003; Dijkstra et al. 2007; Kremer & Rocha 2011; Lambert 2009; Paetzold et al. 2012; Smith et al. 2007; Uribe & Etchepare 2002). These objects are often suspended in the water column and increase habitat complexity and heterogeneity, which is ideal for colonisation by invasive ascidians (Robichaud et al. 2022).

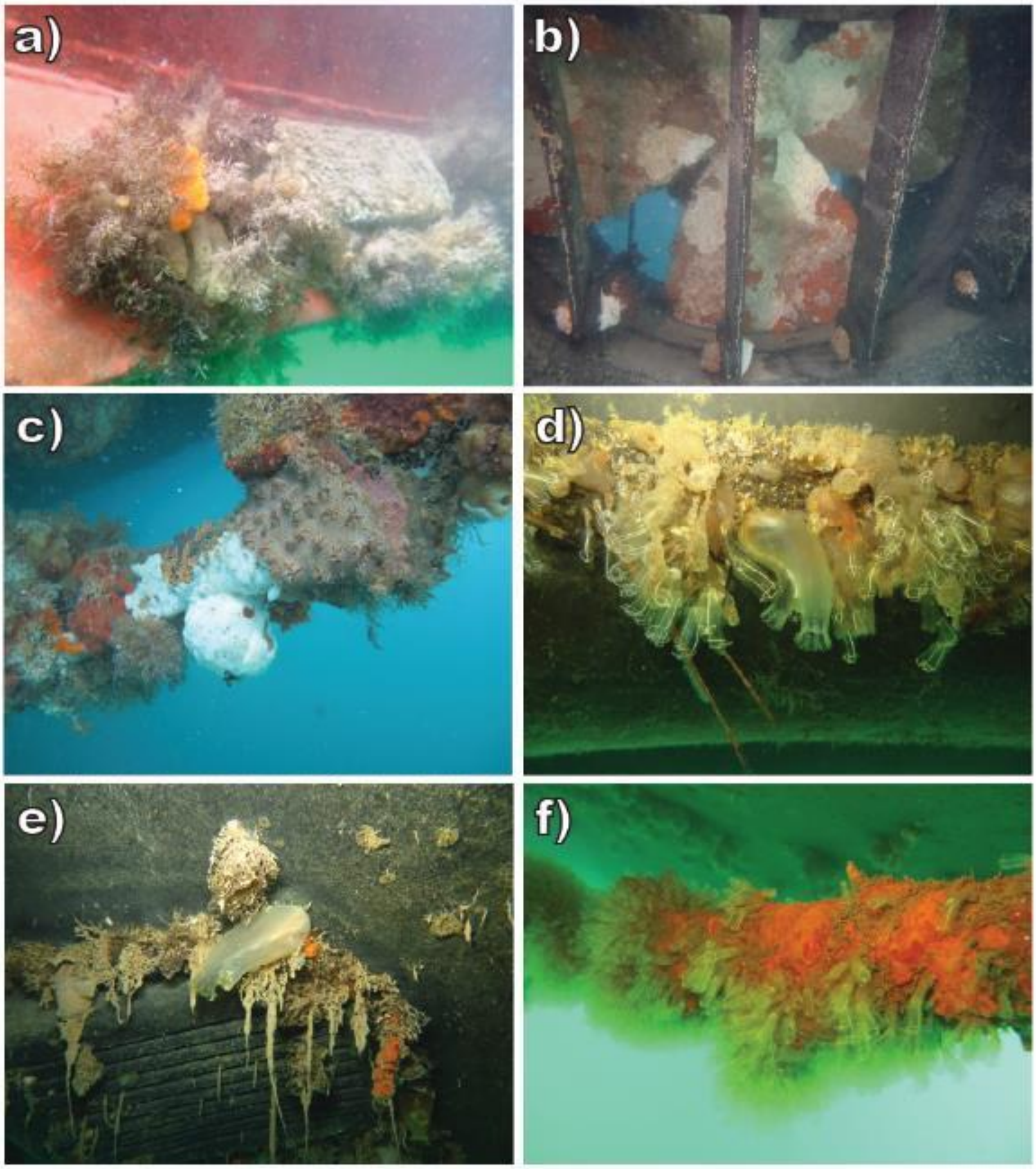
Biofouling on vessel hulls is most likely the primary vector for the long-distance dispersal of invasive ascidians (Aldred & Clare 2014; Daley & Scavia 2008; Forrest 2013; Lambert & Lambert 1998; Lambert 2002, 2009; Pleus et al. 2008). Species such as *Botryllus schlosseri* have most likely been spread via vessels since ancient times (Lambert & Lambert 1998). Even with antifouling paints on vessel hulls, invasive ascidians can attach to areas such sea chests, rudder posts, and weld seams that are challenging to protect (Bridgwood et al. 2014; Daley & Scavia 2008; Deibel et al. 2014).

Photo 5 Examples of ascidian biofouling on different marine structures



Ascidians biofouling on structures. a) *P. pachydermatina* on a wharf piling in Dunedin, New Zealand; b) *P. doppelgangera* on piling at Brighton Jetty, Adelaide; c) *E. elongatum* on a wharf piling in Pukenui, New Zealand; d) *C. lepadiformis* on a marina jetty in Shotley Gate, UK. Sources*:* a) John Barkla; b) Janine Baker; c) Javier Couper; d) Bonnie Caleno.

Photo 6 Examples of ascidian biofouling on vessels



Ascidians biofouling on vessels, including niche areas. a) Ascidians and algae surrounding an anode in Wellington Harbour, New Zealand; b) *Didemnum perlucidum* on a propellor in Western Australia; c) *D. perlucidum*, *Botrylloides* spp. and *Didemnum psammatodes* fouling on a propellor shaft in Lyttelton harbour, New Zealand; d) *Ciona savignyi*, *Diplosoma listerianum* and *C. lepadiformis* encrustation on bilge keel in Tauranga harbour, New Zealand; e) *Ciona intestinalis* and *Botrylloides* sp. surrounding an intake grating in Lyttelton harbour, New Zealand; f) *C. lepadiformis* and an orange sponge fouling a propellor shaft in Tauranga harbour, New Zealand. Sources*:* a) New Zealand Dive and Salvage; b) Franmarine Underwater Services Pty; c) Aquatic Pest Biosecurity, DPIRD; d – f) C. Woods, Earth Sciences New Zealand.

Weather events such as tsunamis may augment the long-distance dispersal of ascidians on fouled objects such as buoys and wharf timbers (Deibel et al. 2014). In the 1920s, sexually mature *P.*

praeputialis may have been introduced from Australia to Chile by rafting (likely attached to flotsam), as well as vessel biofouling and ballast water (Castilla et al. 2002).

Colonial ascidians, particularly didemnids, often overgrow other fouling organisms (Aldred & Clare 2014; Bullard et al. 2007; Smale & Childs 2011). While invasive ascidians are unlikely to die out entirely, colonial ascidians are generally thought to be pioneering fouling organisms that are succeeded by solitary ascidians, and later by other organisms such as bryozoans (Deibel et al. 2014).

Biofouling management

Biofouling can occur on all fixed or mobile structures immersed or exposed to the water. Fouling communities typically comprise of 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, sea stars, 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 hull of vessels or in their internal seawater systems are the main ways that vessels can act as vectors for invasive ascidians.

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 for vessel operators 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, with the benefit of managing potential fouling by marine pest species.

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 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. It should be noted that management of other pathways from areas of existing marine pests may help to minimise the risk of further spread.

Biofouling management

The Australian biofouling management requirements set out vessel operator obligations for the management of biofouling when operating vessels under biosecurity control within Australian territorial seas.

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

Dry-docking and in-water operation principles and recommendations are contained in the Australian anti-fouling and in-water cleaning guidelines - exposure draft. 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. 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
- 1996 protocol to the Convention on the prevention of marine pollution by dumping of wastes and other matter, 1972
  - 2011 Guidelines for the control and management of a ships' biofouling to minimize the transfer of invasive aquatic species (updated in 2023).

The (Commonwealth) *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.

  - recreational vessel.

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

2.2 Ballast

Risk of ascidian dispersal by ballast water

Due to the limited natural dispersal ability of ascidians, with a brief free-swimming larval phase, vessel ballast water (including floating substrates and sediments) is not considered to be a significant vector compared to epibiosis and biofouling (Aldred & Clare 2014; Daley & Scavia 2008; Lambert & Lambert 1998; Lavoie et al. 1999; Simkanin et al. 2016; Zhan et al. 2015). However, given the high fecundity and long reproductive seasons of many ascidians, it is not surprising that ascidian larvae have been found in ballast water (Chu et al. 1997; Daley & Scavia 2008; Lambert & Lambert 1998; Simkanin et al. 2016). There is currently no evidence that larvae can survive, metamorphose and sexually mature within ballast tanks over long oceanic voyages (Zhan et al. 2015). Although unlikely, ballast water should not be excluded as a vector, as it is possible that short (<24 hour) coastal voyages may transport invasive species (Daley & Scavia 2008; Simkanin et al. 2016), but no introductions have been directly linked to ballast water (Zhan et al. 2015).

Ballast water management

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 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 ascidian species as planktonic stages (e.g. gametes, larvae, or fragments), and fouling ascidians 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 that may be transported to other locations (Carlton 1985; Davidson & Simkanin 2012).

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 and aquaculture

Fishing and aquaculture operations can translocate ascidians accidentally with other stock or bait movements, or deliberately by illegal movements of live ascidians for the purpose of establishing a population for cultivation. Ascidians may also be accidently transported on aquaculture and fisheries equipment such as vessels, buoys, ropes, nets, and cages. Although there are strict regulations of live animal imports, there is still some risk of introduction of invasive ascidians 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.

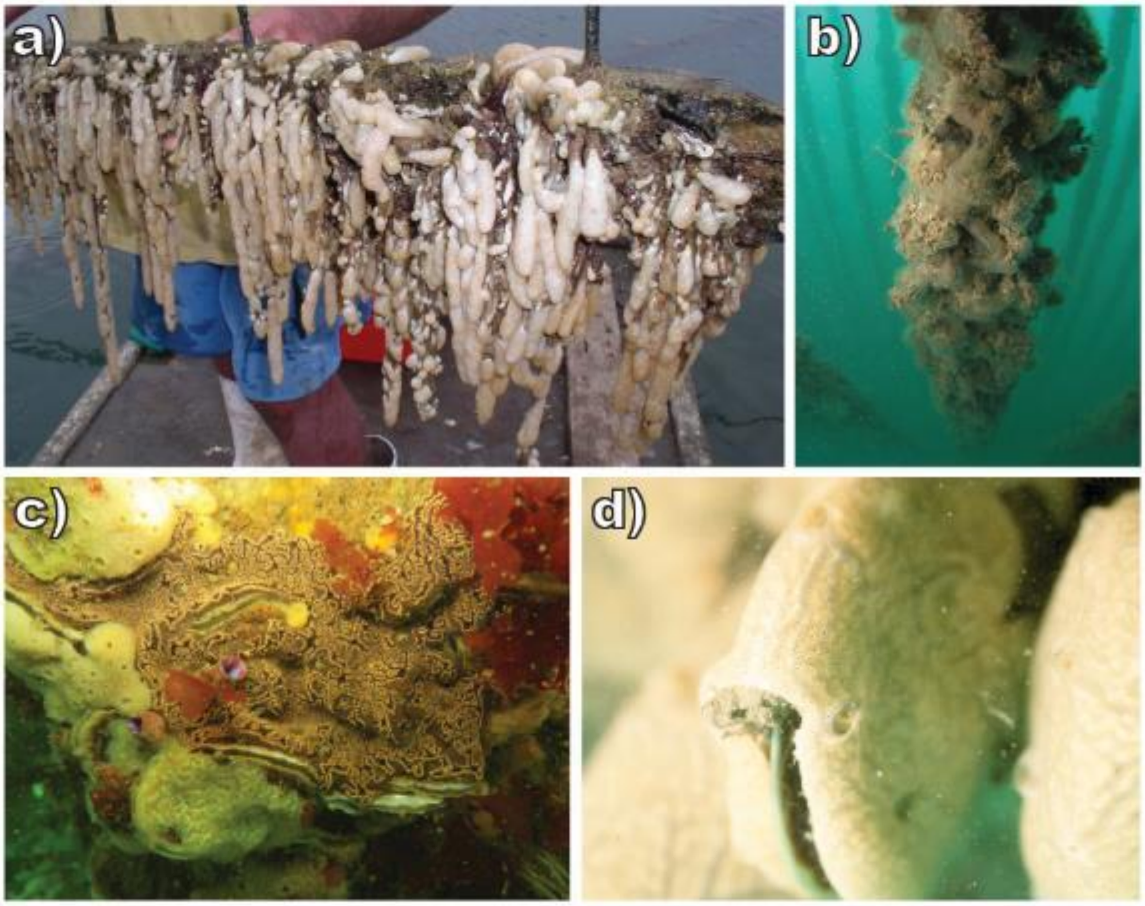
There is risk of marine pest translocation within Australia through domestic trade of live aquatic animals for socio-economic and environmental benefit (DAFF 2020a). The National policy guidelines for the translocation of live aquatic animals have been developed to guide any translocation activity of live aquatic animals.

The ornamental trade is not as significant for the translocation of invasive ascidians 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 ascidians, 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 ascidians that inhabit rocks and other complex structures. Gravel and aquarium water released into waterways may also spread any ascidians (larvae or fragments) present. Import conditions prevent importation of live rock with viable invertebrates (see Australian Biosecurity Import Conditions - BICON).

2.3.1 Biofouling on aquaculture species

Ascidians can settle and grow on other organisms, which significantly augments their dispersal ability and invasive threat (Carman & Grunden 2010; Carver et al. 2003; Dijkstra et al. 2007; Lambert 2009; Paetzold et al. 2012; Smith et al. 2007; Uribe & Etchepare 2002). Many ascidian species grow directly as epibionts on various species of farmed oysters, mussels, and scallops (Fletcher et al. 2013b; Inglis et al. 2013; Papadopoulos et al. 2023; Rocha et al. 2009; Rolheiser et al. 2012; Simkanin et al. 2016; Switzer et al. 2011; Uribe & Etchepare 2002) (Photo 7). By reducing water circulation and competing for dissolved oxygen and plankton nutrition, ascidians fouling equipment and growing directly on bivalves can significantly increase mortality and substantially reduce productivity of aquaculture farms (Carver et al. 2003; Fletcher et al. 2013b; Papadopoulos et al. 2023; Uribe & Etchepare 2002). Both solitary and colonial ascidians compete for space with bivalves, and they dislodge and smother them (Arens et al. 2011; Bullard et al. 2007; Davis et al. 2018; Denny 2008; Fletcher et al. 2013b; Papadopoulos et al. 2023). The low surface pH of some invasive ascidians seems to prevent the settlement of scallop larvae, reducing recruitment (Morris 2009), and molecular evidence indicates that mussels exhibit increased physiological stress from ascidian epibiosis (Papadopoulos et al. 2023). Significant infestations of invasive ascidians increase the maintenance and processing costs for farming (Carver et al. 2003; Fletcher et al. 2013b; Paetzold et al. 2012), notably by forcing equipment to be cleaned before harvesting (Daley & Scavia 2008; Knorek 2018; Switzer et al. 2011).

Photo 7 Ascidians growing on shellfish and aquaculture equipment.



Ascidian fouling on shellfish and aquaculture equipment. a) *E. elongatum* covering oyster equipment from Orongo Bay, Bay of Islands, New Zealand; b) *Diplosoma listerianum* fouling mussel spat lines in Wainui, New Zealand; c) *Botrylloides diegensis* fouling Pacific oysters *Crassostrea gigas* in Bluff Harbour, New Zealand; c) *D. vexillum* encrusting cultured mussel (*Perna canaliculis*) valves in the Marlborough Sounds, New Zealand. Source*:* a – d) M. Page, Earth Sciences New Zealand.

The human transport of stock and equipment, often over large distances, means that aquaculture is a high-risk vector for invasive ascidians (Atalah et al. 2021; Denny 2008; Fletcher 2014; Lambert 2002; Rosa et al. 2013; Simkanin et al. 2016; Zhan et al. 2015). It has been hypothesised that *D. vexillum* was introduced to New England (eastern USA) via the transport of contaminated oyster seed stock during the 1970s (Dijkstra et al. 2007), and its rapid spread elsewhere is potentially linked to traditionally limited decontamination practices in aquaculture (Lambert 2009). In New Zealand, *E. elongatum* has spread among oyster farms, on substrates underneath oyster racks (Morrisey et al. 2009; Page et al. 2011), in the intertidal zone on shell and hard substrata among the native brown seaweed *Hormosira banksii* (Neptune's necklace), and in mangrove habitat. Dispersal since introduction in 2007 is facilitated by larval propagules and by aquaculture activity such as the transfer of brood stock from the east to west coast for conditioning (Mike Page [Earth Sciences New Zealand], pers. comm., 2024). Since some invasive ascidians are rare in wild environments outside of aquaculture facilities, spatial management strategies (e.g. movement restrictions) may act as 'fire breaks' to reduce the transmission and connectivity of invasive taxa (Atalah et al. 2020).

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, tissue fragments, or adults to a new location via natural mechanisms in the environment, such as ocean currents. Characteristics that enable invasive ascidians to be spread via this pathway include having a planktonic dispersal phase (sexual or asexual) or ability to foul floating objects.

Although anthropogenic vectors are the most common mechanism for transporting invasive ascidians over long distances (i.e. international and national scales), once an ascidian 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.

While many ascidian species have mechanisms to reduce self-fertilisation (Gasparini et al. 2015; Jiang & Smith 2005; Sawada et al. 2014), a single solitary individual or colony introduced to a new area has the potential to sexually reproduce via hermaphroditism. Colonial species are especially invasive because they can also reproduce asexually through budding and fragmentation, which can be rapid and prolific, quickly leading to the establishment of a population (Brunetti & Mastrototaro 2004). Generally speaking, only colonial ascidians can reattach to surfaces after dislodgement (Aldred & Clare 2014). Some colonial species can grow into tendril-like morphologies to encourage fragmentation and asexual dispersal (Bullard et al. 2007; Coutts & Forrest 2007; Lambert 2002; Nishikawa et al. 2000; Stefaniak & Whitlatch 2014). Some species also produce dormant buds that are more resistant to environmental stressors (Tobias-Santos et al. 2024).

The duration of the dispersal phase for ascidian larvae is typically limited to minutes or hours, and larvae generally do not travel any further than several kilometres (Aldred & Clare 2014; Atalah et al. 2021; Fletcher 2014; Fletcher et al. 2013c; Forrest 2013; Lambert 2002; Muñoz et al. 2015; Page et al. 2011; Petersen & Svane 1995; Simkanin et al. 2016; Stefaniak & Whitlatch 2014). The regionally invasive solitary ascidian *P. doppelgangera* has a short pelagic propagule duration lasting less than one day, and genetic diversity among non-native populations in southern Australia indicates that there is limited dispersal and gene flow among geographically distant populations (Teske et al. 2014; 2015). Mucus strings and foam are produced by some solitary taxa, such as *Ciona* and *Pyura*, to aggregate and aid the dispersal of their gametes and larvae (Aldred & Clare 2014; Petersen & Svane 1995). A minority of ascidians also have direct developing larvae (Bates 1995), which is likely to further reduce dispersal distances. Higher ambient water temperatures also increase the development rate of ascidian larvae, shortening the larval dispersal phase and causing faster rates of settlement (David et al. 2010).

Broadcast spawning and brooding in solitary and colonial ascidians respectively also influence the impact of dispersal (and gene flow) during incursion events. Solitary and colonial ascidians sampled from the same geographic area can exhibit markedly different population structures and genetic diversity (Ayre et al. 1997). Such genetic diversity may affect the long-term survival and persistence of introduced taxa.

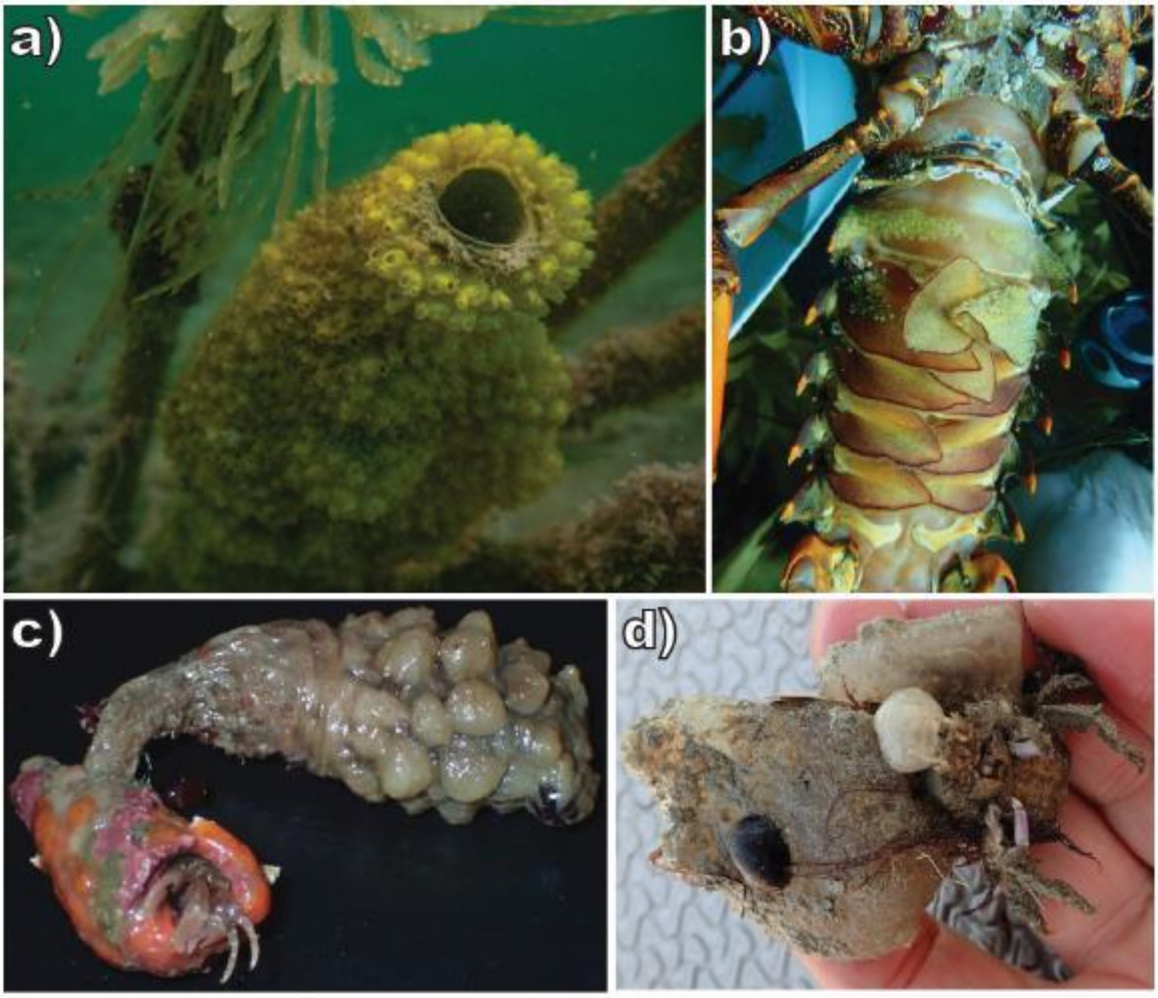
2.4.1 Natural dispersal via biofouling on wild organisms

Both solitary and colonial ascidians may grow on top of other organisms as epibionts, instead of colonising surfaces or objects (Spyksma et al. 2024) (Photo 8). Epibiosis on mobile animals or algae and plants capable of rafting has the potential to increase the dispersal of invasive ascidians. Since these hosts are wild, this is a natural pathway for the spread of invasive ascidians that is not necessarily influenced by human activity.

In the Gulf of St. Lawrence, Canada, ascidian epibionts (*Botrylloides violaceus, B. schlosseri, Molgula spp.*) have been observed on crabs and lobsters (Bernier et al. 2009). Moulting of crustacean hosts is not likely to limit the dispersal of invasive ascidians, given that epibionts should be able to survive and reproduce on discarded exoskeletons (Bernier et al. 2009). *Botryllus schlosseri* has been observed growing on a seahorse in the Black Sea, revealing that ascidians can also sometimes colonise slow-moving fish (Kayiş 2011).

Various ascidian species (*A. aspersa, A. constellatum, B. violaceus, B. schlosseri, C. intestinalis, D. vexillum, D. listerianum, D. perlucidum, M. manhattensis, S. clava, S. brakenhielmi*) have been found to grow on marine plants and algae (Carman & Grunden 2010; Carman et al. 2009b; Simpson et al. 2016). Floating pieces of eelgrass found to have been colonised by some taxa have the potential to travel up to 200 times further than ascidian larval dispersal distances (Carman & Grunden 2010; Carman et al. 2009b).

Photo 8 Ascidians growing on wild organisms



Ascidian epibiosis on wild organisms. a) *S. brakenhielmi* growing on a *Sabella spallanzanii* fanworm near Waiheke Island, New Zealand; b) a *S. brakenhielmi* infestation on a *Jasus edwardsii* lobster in the Hauraki Gulf, New Zealand (Spyksma et al. 2024); c) a hermit crab with an attached *Styela clava* in Lyttelton Harbour, New Zealand; d) solitary and colonial ascidians attached to the masking crab *Notomithrax* sp. in Lyttelton Harbour, New Zealand. Sources*:* a) Shaun Lee; b) Nick Shears, Leigh Marine Laboratory, University of Auckland; c, d) C. Woods, Earth Sciences New Zealand.

Case example: Natural dispersal ability of Didemnum

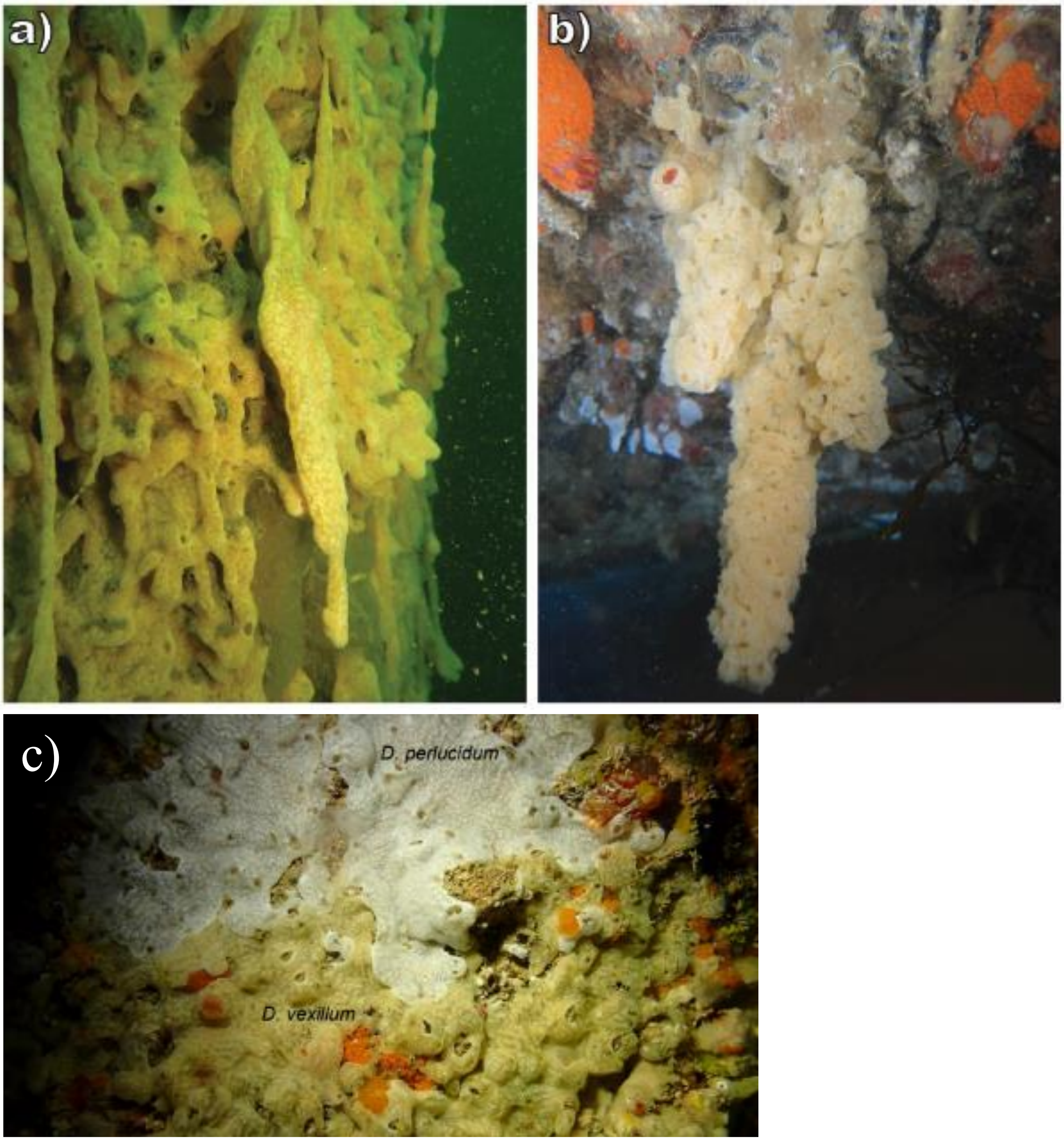
Didemnum perlucidum and *D. vexillum* are colonial ascidians made of hundreds to thousands of small zooids less than 2 mm long, all interconnected by canals (Daley & Scavia 2008; Muñoz et al. 2015). Because zooids can bud asexually, colony growth can be exponential, giving these species a competitive advantage over adjacent sessile filter feeders and the ability to over-grow and smother (Stefaniak 2017).

During sexual reproduction, larvae are brooded in the common test of the colony and released seasonally to disperse (Bullard et al. 2007; Daley & Scavia 2008; Lambert 2009; Stefaniak & Whitlatch 2014). Larvae are mobile for 2–36 hours and can potentially disperse over distances of as great as 1 km (Fletcher et al. 2013c; Stefaniak & Whitlatch 2014). The seasonal reproduction of *D. vexillum* in New Zealand extends for up to 9 months and is strongly correlated to water temperature (Fletcher et al. 2013a). Colonies have been found growing in waters between –2°C to 24°C (Daley & Scavia 2008; Valentine et al. 2007a), although reproduction and growth typically peak in warm summer months at temperatures >12°C (Fletcher et al. 2013a; Ordóñez et al. 2015). In Western Australia, while larvae have been found in colonies of *D. perlucidum* during winter, no new colonies appear to settle during those cooler months, and reproductive activity seems to peak in summer (Muñoz et al. 2015). After settlement and metamorphosis, *Didemnum* zooids can reach sexual maturity within only a few weeks (Daley & Scavia 2008; Stefaniak & Whitlatch 2014).

Like many invasive ascidians, *Didemnum* species frequently foul vertical surfaces and suspended objects, but they can also colonise the seafloor and grow as mats over pebble and gravel substrates (Smale & Childs 2011; Valentine et al. 2007b) (Photo 9a). Colonies seem to require hard substrates to survive though, and *D. vexillum* has not become established in exclusively soft-bottom habitats (Bullard et al. 2007). Fouling and mats of *Didemnum* spp. typically overgrow other fouling organisms (Bullard et al. 2007; Smale & Childs 2011).

Fragmentation is also an important dispersal mechanism for *Didemnum* species. Colonies can grow into rope or tendril-like shapes on floating structures, which helps to release fragments into the water column (Bullard et al. 2007; Coutts & Forrest 2007; Stefaniak & Whitlatch 2014) (Photo 9b). These fragments, often with larvae, remain viable and can reattach to surfaces during extended periods of contact, thereby increasing the dispersal distance of reproductive populations (Coutts & Forrest 2007; Muñoz et al. 2015; Stefaniak & Whitlatch 2014). Drifting tendrils, have been observed to be densely packed with brooded larvae, further increasing dispersal potential (Stefaniak & Whitlatch 2014).

Photo 9 *Didemnum* spp. tendrils and mats



Photographs of tendrils and mats in *Didemnum* spp. a - b) *D. vexillum* with tendrils, c) mats of *D. perlucidum*, *D. vexillum* (labelled. Sources*:* a) C. Woods, Earth Sciences New Zealand; b) and c) DPIRD, WA.

3 Preventing and monitoring spread

Preventing spread and monitoring efforts enhance the chances of containment and eradication. The likelihood for eradication of an incursion by an invasive ascidian will increase with early detection and rapid action. In areas with very limited suitable substrate for attachment, containment may be possible for some species with low dispersal capability. Where surveys indicate a widespread incursion, management strategies may need to focus on limiting further spread and reducing population density; vital to this is vessel and vector management. In all cases, intensive public consultation and education is essential to ensure support and/or compliance with response actions.

This section covers invasive ascidian 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 an invasive ascidian 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
- transition to management 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. 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, manage expectations, support compliance with any regulations, and to encourage participation in surveillance activities and reporting.

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

The affected jurisdiction may establish an Incident Management Team (IMT), in which a Public Information function may 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 or zoning, 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 investigations provide additional information. They may end up being permanently implemented to minimise risk of spread or impacts in a long-term management program. However, where possible a time frame is best included in communications materials where actions such as quarantine may be disruptive.

When a suspected invasive ascidian 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. Port and marina operators, aquatic industries, 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 ascidian and will also identify areas where the ascidian is not detected. As part of the investigation phase, delimitation informs feasibility of eradication and areas to target for eradication or control and management. Delimitation is needed to inform the next steps of a response or determining the current status of a marine pest (present or absent). In some cases, delimitation may take over one year to capture seasonal appearance of invasive ascidians.

For more information on delimiting an incursion see Section 4.1.

3.1.4 Tracing an incursion

Tracing is used to determine the mechanism and pattern of the spread of an invasive ascidian and may include trace-forward and trace-back. Tracing back is used to discover where an incursion may have originated from and identify potential additional outbreak sites within Australia. The first location to have the detection of an invasive ascidian may not be the original site of introduction. Tracing is crucial to defining and modifying the dimensions of the specified areas defined in Figure 5.

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 the likely vector pathway of introduction 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 or colony fragments (Burgman et al. 2013; Hauser et al. 2016). The use of DNA-based methods may be able to identify both source and connected populations and areas of provenance (Roux et al. 2020).

Elements of demography of the invasive ascidian populations may be inferred from the size or age distribution within the population and reproductive state of ascidians 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/or 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). 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 ascidian's species identity and should attempt to identify all potential vectors present at the outbreak site. Invasive ascidians may be hard to distinguish from native species, and identification will need to be 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 ACEBO 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. An initial PIDS may be incomplete and will be adjusted as more is found about the pest through surveillance and tracing activities.

Potential vectors for invasive ascidians are discussed above 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 to assist with assessment of all potential spread risk pathways, and to minimise 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 ascidians. That appendix has sample handling and preferred narcotising, fixation, and preservation techniques for ascidians. 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 ascidian is likely to be present, the combat jurisdiction should communicate the findings of the investigation to CCIMPE and seek additional input including recommended course of action to manage the risk of spread.

During the alert phase, an incident management team (IMT) may be appointed to coordinate the combat jurisdiction response, which may include operations to confirm the identification of the suspected invasive ascidian and the likely extent of an incursion. 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 sites, as appropriate
- managing water movements where possible
- promoting awareness of methods to report sightings of the pest and access to general information
- tracing potential vectors that have left the affected site
- hydrodynamic modelling, and tissue fragment analysis for colonial ascidians, to determine potential spread of larvae and gametes
- redirecting vessels that have already left the site to appropriate sites for inspection and/or decontamination if appropriate and within legislative capabilities
- 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 instance, 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 ascidian 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 ascidian 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 ascidian, then movement restrictions implemented in the investigation phase should remain in place and amended as appropriate to reflect emerging information.

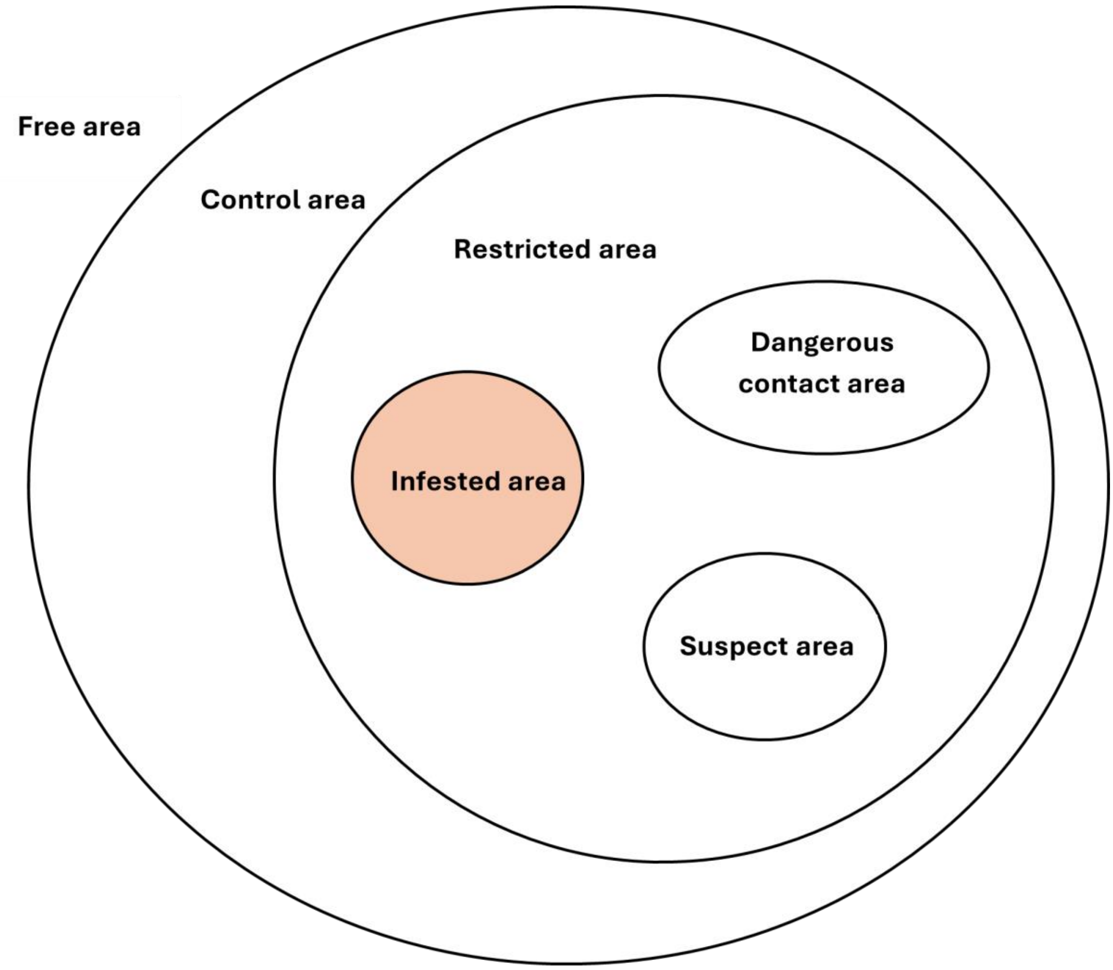
Quarantine restrictions put in place could be categorised as below to direct response actions (Figure 5):

- 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 5 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 ascidians 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 may not be appropriate. 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, including the shape of each area.

Movement restrictions may include requirements placed upon:

- 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 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 ascidian is unlikely to be eradicable, the combat jurisdiction should implement interim containment measures to prevent translocation from any infested waterway to minimise the risk of the pest being spread from the affected area.

CCIMPE will consider technical feasibility to eradicate based on evidence and data provided by the combat jurisdiction. If CCIMPE determines that eradication is not feasible, CCIMPE will provide this 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 the 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, and transition is considered a component of the response.

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.

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

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 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.

Where appropriate, 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 biofouling and Section 3.3.2 on ballast water management for 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 the 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 should commence once operational objectives have been achieved, or otherwise in accordance with advice provided by CCIMPE and agreed by the NMG. communicated

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.

3.2 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, marine infrastructure, and habitats in the receiving environment[5] should be risk assessed and if they were in the restricted or control areas during the time the marine pest was suspected to have been present, management requirements determined.

If this risk assessment determines any vessels, marine infrastructure, surrounding habitat, and other vectors to present at a high or unacceptable risk, recommendations may include requirements for inspection and treatment:

- risk management measures could include inspections of vessels
- infrastructure or surrounding habitat
- vessels to remain in the control area, treatment of infrastructure or vessels,

For example, a recently cleaned vessel will have lower likelihood of harbouring invasive ascidians than a heavily fouled vessel (MPSC 2021) and present differing level of risk.

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 should 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.

3.2.1 Vessel inspection

The Australian biofouling management requirements set out vessel operator obligations for the management of biofouling when operating vessels under biosecurity control within Australian territorial seas. Ascidians 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 guidelinesAustralian anti-fouling and in-water cleaning guidelines - exposure draft and International Maritime Organization (IMO)

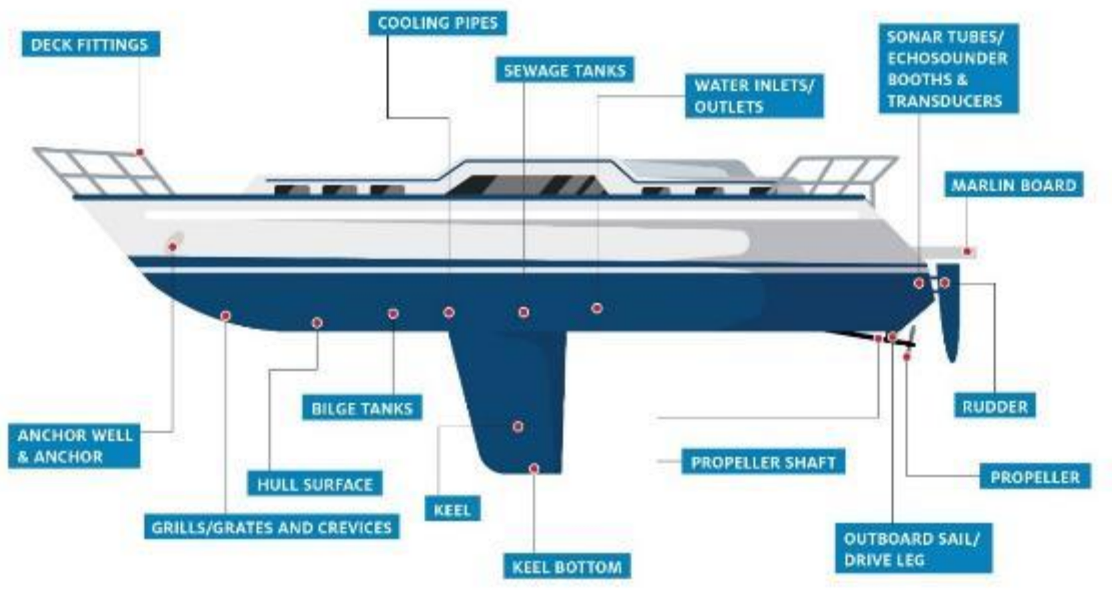
[5] Marine infrastructure and habitats in the receiving environment may be naturally occurring or man-made.

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 ascidians.

Critical inspection areas for vessels less than <25 metres long (Figure 6**Error! Reference source not found.**) 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 6 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



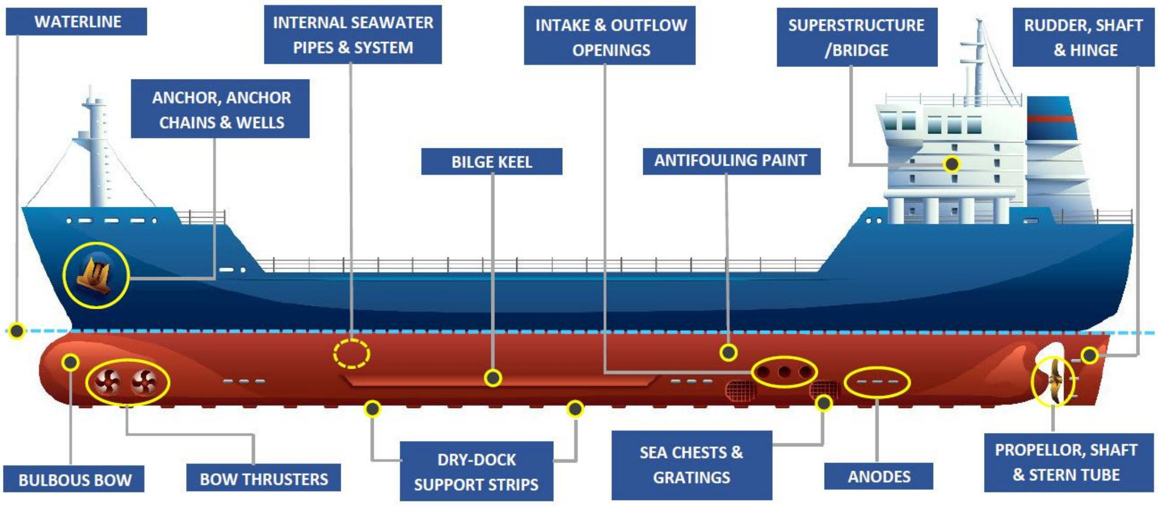
Source: Floerl (2004)

Critical inspection areas are similar for vessels longer than >25 metres (Figure 7), 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 7 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 Inspections of marine infrastructure and habitat

Surveillance for invasive ascidians should include 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 ascidians. For example, the infrastructure that supports vessel operations (e.g. boat harbours, marinas, slipways, recreational boating mooring areas, and fishing ports/bases) provide 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 the 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 ascidian 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 invasive ascidians 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. The information below summarises management recommendations for different types of vectors which may translocate invasive ascidians.

Case example: Management of an infested vector during a response
During the Australian response to *Didemnum vexillum* several methods were applied to treat the underwater areas of an infested vessel, including the hull and niche areas. A closed circuit in-water cleaning system with hard tools and suction nozzles was used to remove biofouling, including *D. vexillum*, from the vessel's hull and niche areas. The captured material was passed through 10 µm particulate filters and ultraviolet (UV) light to ensure disinfection. Blanking plates were also installed by divers to mechanically seal and isolate sea chests for chemical treatment. Once sealed, sea chests were de-watered by air compression and subsequently flooded with a Rydlyme® and freshwater solution, followed by a waiting period to ensure complete mortality. In addition, the vessel underwent dry-docking several months later, an effective method of desiccation by air-drying. Table 3 below summarises management recommendations for different types of vectors which may translocate invasive ascidians.

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

Vector	Management action options
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, e.g. bilges, anchor wells, live wells/bait tanks, scuppers) 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, e.g. bilges, anchor wells, live wells/bait tanks, scuppers) Manage ballast water Educate operators and service agents of risk
Recreational craft (e.g. jet-skis and kayaks)	Remove from water and clean internal and external submerged surface Treat and/or clean and dry internal seawater systems and wetted surfaces Educate users and service agents of risk

Vector	Management action options
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 and use an effective method for decontamination Educate users and service agents of risk
Fouled aquaculture equipment	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 freshwater and air dry for at least 48 hours, preferably a significant portion of this time in direct sunlight Remove from infested area Educate users and service agents of risk Prevent waste water re-entering any waterway
Buoys, pots, floats, and fenders	Restrict movement from the control area Treat and/or clean and dry (as above) Prevent waste water re-entering any waterway Educate users and service agents of risk
Water, shells, and organisms for bait/burley 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 of disease, for example, viruses may also be carried via the gut, feathers, feet and bill of piscivorous birds (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 Australian anti-fouling and in-water cleaning guidelines - exposure draft 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 ascidian, prevention of entry, treatment, or vessel cleaning before entry to a port are the most effective management options. Where vessel 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. Sites of infestation need to be known to avoid dislodging pests into the water when lifting the vessel.

Australian dry dock facility information can be obtained from the National Maritime Centre (NMC).

In-water cleaning may be subject to consideration by biosecurity, environmental and port authorities, and may require referral under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) (e.g. in instances relating to Ramsar-listed wetlands, National Heritage places etc.). If the activity does not require referral under the EPBC Act, the activity must be self-assessed using the Australian anti-fouling and in-water cleaning guidelines - exposure draft

Decision support tools. 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 In-water cleaning in Australia webpage.

The *Biosecurity Act 2015* can be used in the absence of appropriate state or territory legislative powers and may be used in 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 ascidians may 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 mooring blocks, pontoons, floats, fenders 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 or 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).

Care should be taken when removing vessels or structures fouled by colonial ascidians, because tissue fragments may disperse and asexually reproduce within water when disturbed. There is also a risk that ascidians dislodged during haul-out or vessel cleaning may remain viable and 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.

Air-drying is an effective treatment for ascidians (see Section 5.3.1.1). 48 hours of air-exposure is generally recommended (Deibel et al. 2014), although some solitary ascidians such as *Styela* spp. have exceptional tolerance to desiccation and require two weeks exposure for 100% mortality (Hillock & Costello 2013). Efficacy depends upon ambient temperature and humidity and exposure to direct sunlight, and treatment times can be significantly reduced by combining air-drying with chemical treatments and manual removal (Deibel et al. 2014; Inglis et al. 2013). High-pressure water blasting (≤ 8000 psi) is also an effective method for removing ascidians from hauled-out vessels and structures (Coutts & Forrest 2005; Inglis et al. 2013). However, water blasting is less effective for treating recessed and niche areas (Inglis et al. 2013). See Section 5.3.1.7 for further information.

Antifouling paints, correct for the operational profile, which can be applied to vessels and structures in dry docks, can be effective for preventing future ascidian biofouling. Experiments using settlement plates indicate that antifouling paints keep ascidian fouling at low levels for at least 12 weeks of continuous submersion (Darbyson et al. 2009). When applying chemical treatments though, users should be aware that the pH changes associated with strong acids and alkali are likely to affect the efficacy of existing antifouling paints (Morrisey & Woods 2015).

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

Some ascidians are robust organisms capable of tolerating extreme environments. Internal seawater systems of vessels should be cleaned to the greatest extent possible with:

- chemical treatment
- thermal treatment.

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 relevant environmental protection authorities and legislation or by the waterway or port managers. Refer to Section 5.3.2.1 regarding permits to chemically treat waterways. Thermal treatment is non-selective but residue-free, with localized effects, and can be applied via hot water or engine heat to substrates, piping, or ballast water using methods like immersion or hydrodynamic cavitation. Refer to Section 5.3.1.9 for further information on thermal treatments.

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 include in-water cleaning as an option for removing biofouling, provided risks are appropriately managed and can be supported by relevant jurisdictional authorities.

A suite 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 (Inglis et al. 2013; Morrisey & Woods 2015), but the dislodgement of fragmented colonial ascidians remains a significant risk for spreading invasive taxa (see Section 5.3.1.6). Capture of particles >10µm is recommended as a measure to reduce risk of potentially viable fragments or gametes escaping.

In-water encapsulation using impermeable plastic wrapping is a highly effective treatment for invasive ascidians, where possible (see Section 5.3.1.2). Commercially available devices can be used to encapsulate small vessels and pontoons, which can be effectively combined with chemical treatments (Keanly & Robinson 2020; Morrisey et al. 2016; Roche et al. 2015).

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 In-water cleaning in Australia 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 usually structurally complex, in some cases 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 ascidians can be attractive habitats for other marine pests. Biofouling of sea chests, internal pipework, bow thrusters 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 ascidians include chemical and non-chemical methods. There are considerations for effective in-water treatment. For instance, a key element of in-water treatment 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.

For non-chemical treatments, only thermal stress can feasibly be applied to pipework and niche areas and be effective within 48 hours. Heat treatments have not been tested significantly against ascidians (see Section 5.3.1.8), but prolonged exposures to 40 or 50°C heated water may be sufficient to eliminate some ascidians in sea chests (Sievers et al. 2019).

For chemical treatments, lime and acetic acid are generally considered to be most effective chemical treatments for invasive ascidians (Pleus et al. 2008) (see Section 5.3.2). Acetic acid and commercial descaler formulations can be effective against general fouling assemblages within sea chests within 48 hours (Cahill et al. 2019; Cahill et al. 2021), but no studies have currently focused upon ascidians. Refer to Section 5.3.2.1 regarding approvals to chemically treat waterways, which may require additional approvals from other jurisdiction agencies. In the NT, quaternary ammonium compounds in a commercial detergent for ten hours can be highly effective general assemblage disinfectants and neutralised ascidians in treatments of mixed infections including *Didemnum perlucidum* in niches (NT Department of Agriculture and Fisheries 2025, pers. comm., 29 July).

An important consideration for chemical treatments is its risk to the environment and operator, and its efficacy. Acetic acid and chlorine are considered safe to use within the marine environment; however, their efficacy needs to be determined prior to use. 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. Osmotic treatments are unlikely to be effective to remove ascidians from niche areas as they generally require prolonged exposure periods for high mortality rates (see Section 5.3.2.3).

Physical removal of a marine pest from niche areas is not always possible or feasible. There is a significant risk of inadvertently releasing invasive ascidians into the environment without sufficient measures to ensure that no viable material can escape (including tissue fragments for colonial ascidians). Since encapsulation and smothering are highly effective (see Section 5.3.1.2**Error! Reference source not found.Error! Reference source not found.**), sealing pipes and grates to cause anoxia and ammonium toxicity may be an effective physical treatment for invasive ascidians in sea chests. Research involving other marine pests indicates that using oxygen scavenging chemicals to accelerate deoxygenation may increase efficiency, but efficacy should be monitored (Cahill et al. 2021).

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* regulates the discharge of ballast water and ballast tank sediments in Australian waters. The Act prohibits the discharge of unmanaged ballast water within Australian seas (within 12 nautical miles of any land mass or in water less than 50 metres deep) (DAFF 2023b), unless granted an exemption by the Director of Biosecurity. Vessels intending to discharge ballast water in Australia must apply for permission via the Maritime and Aircraft Reporting System and receive a valid Biosecurity Status Document prior to any discharge. The Act also prohibits the discharge of ballast tank sediment within Australian seas. 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 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 unless a valid exemption applies.

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 ascidians 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 in the case of shellfish or crustaceans. 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.

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 marine pests 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 ascidian species, or potentially other pest species and parasites (Goedknegt et al. 2018). Similarly, invasive ascidians can settle on the shells of cultured bivalves and on aquaculture equipment. See Section 2.3.1 for examples of ascidian biofouling on aquaculture species.

Aquaculture stock can be treated by:

- manual removal/destruction
- detergents
- osmotic treatment.

Different marine pests vary in their susceptibility to manual removal or exposure to toxicants. Ascidians are less likely to withstand desiccation or toxicants than some cultured species (e.g. bivalves). 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 off 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 a 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 ascidians. 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 and buoys
- 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 ascidian 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 ascidian, 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 sometimes used interchangeably with surveillance.
- **Delimitation –** Delimitation is a form of surveillance that establishes the geographic extent of an area infested by, or absent of, an invasive ascidian 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 of an incursion

After the detection of an invasive ascidian, 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 infested area(s) should be implemented to reduce the potential spread of the invasive ascidian. 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 ascidians 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 ascidian was initially detected:
  - how long the ascidian has been present at the site before it was detected
  - the dispersal characteristics of the ascidian, 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.
- ascidian biology, such as survival reproductive rate and current stages of reproductive development
- ascidian habitat, such as distribution and suitability of potential habitats around restricted areas and control areas
- survey method, such as survey sensitivity (factoring detection method sensitivity, including ascidian 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 habitat suitable for the invasive ascidian. 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 ascidian 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 types of 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 ascidian 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. Marinas record clients and vessel movements.

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 a *Mytilopsis sallei* mussel 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 ascidian 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 hydrodynamics of specific locations such as ports and can be quite expensive to run (see Summerson, Hester & Graham 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 (Brown et al. 2013; Thompson 2004). 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 ascidians) 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. Early detection is facilitated by robust, reliable, and practical surveillance techniques tailored to detect these pests.

The Marine Pest Sectoral Committee (MPSC) has 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 of considerations for designing a surveillance program to detect invasive marine ascidians, and key types of surveillance.

4.2.1 Developing surveillance programs

When designing and planning a surveillance program for invasive marine ascidians, 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
- Data management – includes data collection, collation, curation and sharing of information to maintain sample and data collection integrity
- 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 ascidian 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, industry agencies or contractors. 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 excluding active surveillance. General surveillance is observer initiated. An example is a report of a suspected invasive ascidian by a member of the public during recreational diving. 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 variations. Surveillance can be done before, during, or after an emergency response to an invasive ascidian 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, cultural value. Targeted surveillance should 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 ascidian 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 ascidian under surveillance.

If an invasive ascidian is successfully eradicated, ongoing targeted surveillance is still crucial as there is always a risk of re-introduction. If an invasive ascidian 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 further reducing its spread, including localised eradication attempts.

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 surveillance, including for delimitation, of invasive ascidians. 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 ascidians 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 approaches can be rapid and cost-effective tools for the surveillance and identification of invasive ascidians. 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. The sensitivity of molecular surveillance techniques depends on the sampling method used as well as on the molecular analysis. Sensitivity may vary seasonally, particularly for methods targeting larval stages or shed DNA in water, due to reproductive seasonality or other factors influencing shedding and decay rates of environmental DNA (eDNA). Molecular methods detect DNA, which may be non-viable or have been advected away from source populations. Further investigation is typically needed to determine source and viability of the material.

For molecular methods to effectively support marine pest management, marker/DNA probe selection, assay validation, sampling procedures, and approaches for interpretation of molecular surveillance 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

Ascidians can be physically collected for molecular analysis, with the main methods of tissue sampling being physical removal of ascidians from underwater structures and vessels by divers, or removal from intertidal structures during visual searches and on-land inspections. Ascidians growing on settlement plates may also be scraped off and later preserved (see Appendix E). For microscopic material, such as ascidian gametes, larvae, fragments, or shed DNA, then plankton tows and filtered water samples can be used. Preservation of the ascidian in either ethanol or formalin will depend on whether molecular analysis or morphological identification will be used . See Sampling processing from settlement arrays in Appendix E for further information.

Molecular methods for ascidian 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 sensitivity and specificity of current technologies for detection. Validation of assays is important to ensure that assays applied do not cross-react with closely related species in a genus, especially for assays developed outside Australia, and that the target is reliably detected.

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 is usually sufficient to identify an invasive species with high reliability. It is recommended to amplify high-copy genes, such as mitochondrial cytochrome c oxidase subunit I (COI), 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, revealing the most likely identity of the sampled ascidian. 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 numerous genetic lineages of ascidians are still taxonomically undescribed (Simpson et al. 2017; Temiz et al. 2023; Westfall et al. 2020; Zhan et al. 2015). Likewise, metadata for sequences are often poorly maintained in sequence databases, which can result in misclassification – as reported for *Botrylloides* spp. (Temiz et al. 2023; Viard et al. 2019). 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 ascidian taxonomic experts who can assess species identity through ascidian zooid morphology. For lists of available experts contact the MPSC Secretariat at mpsc@aff.gov.au.

4.3.1.2 Molecular delimitation and surveillance

Molecular methods are used for delimitation and surveillance by testing environmental samples (e.g. eDNA water samples or plankton samples) collected from a defined area to identify the spatial boundaries of an incursion (Scriver et al. 2024a). This in turn, can assist in the prioritisation of approaches for containment, eradication, and control of ascidian populations. An invasive ascidian may be present at low population densities and have a heterogeneous 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. 2010b; Darling & Frederick 2018; Darling et al. 2017; Darling & Mahon 2011; 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, as well as viable but non-culturable microorganisms (Darling & Frederick 2018).

In surveillance and delimitation, usually one species or taxon will be targeted, therefore species-specific approaches such as qPCR and 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 species DNA 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 has 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 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) has developed a sample number calculator for surveys using plankton tow samples and molecular testing. This calculator assumes a specific plankton tow method (100 m surface tows with a 50 µm net) but can be used to calculate sample numbers for any molecular test with quantified diagnostic sensitivity (= likelihood of detection where target DNA is present).

Australian-developed qPCR assays are currently available for some invasive ascidian species, including *Didemnum perlucidum, D. vexillum* and other *Didemnum* species (Ellis et al. 2023; Simpson et al. 2017, Simpson et al. 2018), and for *Ciona intestinalis* (Bott & Giblot-Ducray 2011). These assays were laboratory validated as part of their development. The assay for *Ciona intestinalis* has been partially field validated for plankton sampling (Deveney et al. 2017) but not fully operationally validated. The available assays for *D. perlucidum* and *D. vexillum* have been operationally validated in settlement plate samples (Wiltshire et al. in prep). In Canada, PCR assays have been developed for *B. violaceus*, *B. schlosseri*, *C. intestinalis*, and *S. clava* (LeBlanc et al. 2020). qPCR and ddPCR assays have also been developed for *C. intestinalis* in South Korea (Bae et al. 2023a) and *S. clava* in New Zealand respectively (Scriver et al. 2024b). These overseas assays have undergone laboratory and, in some cases, initial field assessment, but are not operationally validated. Even where operationally validated assays from overseas are available, PCR assays used in Australia should still be field validated for Australian conditions because of the potential for cross-reaction with native species (the majority of which have not been sequenced). Operational validation is specific to sample type, hence, additional validation may be needed where assays are used in a different sample type. Molecular information including the current level of assay validation can be found for some of the 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-throughput 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; Pochon et al. 2017). 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. HTS approaches are generally less sensitive that PCR-based methods for detections of specific taxa, and may also be less specific due to incomplete databases and the inability of some barcodes to distinguish closely-related species. Managers should be aware that HTS results take considerably longer compared with qPCR/ddPCR due to requiring a multistep process for amplification, sequencing, and bioinformatic processing.

In aquatic environments, detection probability of free DNA, as captured in water samples, 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). Detection in plankton is influenced by seasonal reproductive periods and by both active and passive larval movements; planktonic detections may also include shed adult cells or free DNA adsorbed to particles (Wiltshire 2021). Positive molecular detections of target DNA in these sample types 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 if available to improve temporal surveillance resolution and assist in trace-back activities.

Settlement arrays are a common surveillance method, and can also be tested by molecular methods. Unlike water or plankton samples, material present on settlement plates is known to be from the sample location and viable. Settlement arrays need to be deployed across the appropriate reproductive season, however, and typically for several months, to capture target organisms. This sample type is therefore not well suited to rapid response or delimitation surveys, although it is useful for monitoring and potentially early detection.

A combination of molecular methods and traditional methods (e.g. morphological identification or molecular analyses of material from settlement arrays or visual surveys) are often used for marine pest surveillance to increase the confidence of pest presence. There are pros and cons to both approaches. Traditional surveillance methods can help confirm inconclusive molecular results, but are often more expensive and time-consuming to deploy and usually require taxonomic expertise. Molecular methods can confirm morphologically ambiguous species or multiple species, and can cover large spatial areas at relatively low cost. However, positive molecular detections may be the result of false positives which can occur due to a lack of assay specificity or sample contamination, including ballast or wastewater bearing non-viable material. Sequencing approaches can sometimes be applied to confirm assay results where assay performance is not fully validated. Understanding of related local species will also assist in assessing possibility of cross-reactivity where tests have not yet been validated in Australia. 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). Both molecular and traditional surveillance methods can lack the necessary sensitivity to confirm occurrences of species at low abundances, which is also why both methods are often paired during surveillance.

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 ascidians. 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 sexual 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 detection 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 ascidians. 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).

Draft genome assemblies have been produced for a growing number of invasive ascidians, including *B. leachii* (Blanchoud et al. 2018), *C. intestinalis* (Dehal et al. 2002), *C. robusta* (Bae et al. 2023b), *D. vexillum* (Simpson et al. 2017), *S. clava* (Wei et al. 2020), and *B. violaceus* (Bae et al. 2023a; Bae et al. 2023b; Bott & Giblot-Ducray 2011; Bott et al. 2010a; Deibel et al. 2014; Ellis et al. 2023; LeBlanc et al. 2020; Mastrototaro et al. 2019; Simpson et al. 2017; Smale & Childs 2011; Smith et al. 2007; Temiz et al. 2023; Viard et al. 2019; Westfall et al. 2020; Zhan et al. 2015).

For invasive ascidians, short-range PCR sequencing (as used for Species identification and confirmation) is suitable for identifying broad biogeographic patterns, such as the diversity of genera across countries (Rius & Teske 2013; Temiz et al. 2023; Teske et al. 2011; Viard et al. 2019; Zhan et al. 2015), or among populations of a single species over large geographic areas (Dias et al. 2016; Goldstien et al. 2011; Pineda et al. 2011; Stefaniak et al. 2009; Stefaniak et al. 2012; Torkkola et al.

2013). Microsatellite markers have provided sufficient resolution for more detailed population genetic investigations of invasive ascidians (Ben-Shlomo et al. 2010; Dias et al. 2021; Lin et al. 2017). Genome-wide sequencing methods such as genotyping-by-sequencing provide cost-effective, high-resolution data for more detailed and confident estimates (Vaux et al. 2023), although only *D. vexillum* has currently been investigated using such methods (Casso et al. 2019a; Casso et al. 2019b).

Case example: Insights from the population genetic diversity of Pyura doppelgangera in Australia
Pyura doppelgangera is native to Tasmania but is regionally invasive to mainland Australia and New Zealand (Teske et al. 2014; Teske et al. 2015). Researchers investigated genetic diversity among native and non-native populations in Australia using microsatellite DNA markers (Teske et al. 2014; Teske et al. 2015). Decreasing genetic diversity with geographic distance among non-native populations in southern Australia indicated that the species has limited dispersal ability (Teske et al. 2015). Using population genetic data, researchers also estimated divergence dates among native and non-native locations, which indicated that incursions occurred during the period of European settlement (Teske et al. 2014).

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 (Atalah et al. 2020; Bridgwood et al. 2014; Coutts & Forrest 2007; Deibel et al. 2014; Gewing et al. 2017; Grey 2009; McCann et al. 2013; Simkanin et al. 2012; Simpson et al. 2016). Divers and ROVs may be used for both surveillance activities and delivering treatments for invasive ascidians during instances when these methods are deemed most appropriate.

To collect evidence of invasive ascidians, 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 recognition. Many ascidian species, including invasive *Didemnum* spp. can also not be reliably identified visually or by photograph alone. Divers can collect ascidian specimens by hand, which can be identified using 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 confidence if visual techniques are inadequate.

Divers can be particularly effective at detecting invasive ascidians that tend to aggregate around complex structure such as jetty pylons. However, the ability to observe an invasive ascidian while diving relies heavily on water visibility, identification training and identifiability of the target, safety, and search techniques. Divers can use touch quite effectively to detect some ascidians in inaccessible niches.

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 of 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 may exceed prices >$35,000 AUD (Ellard 2021).

ROVs were deployed as part of delimitation surveillance of Department of Defence assets in Sydney Harbour, NSW, during the Australian response to *Didemnum vexillum*. Similarly, NSW DPIRD engaged a contractor to undertake ROV surveys on artificial structures identified as high priority locations in the greater Sydney Harbor.

Divers are regularly engaged in detection and treatment of marine pests on visiting vessels as well as 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
- wrapping and encapsulation
- smothering
- osmotic treatment.

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. Given that most ascidians 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)
- allow estimation of time of settlement
- 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.

A 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 ascidians, 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 and reproductive timing.
- Arrays need to be deployed for weeks to months to collect material, limiting usefulness of the method for rapid response or delimitation surveys.

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 ascidian (Floerl et al. 2012). The species' biology and life habit will also influence settlement onto arrays.

Various designs of settlement arrays have been used for marine pest surveillance (Floerl et al. 2012; Sutton & Hewitt 2002). Refer to Appendix E for a summary of potential settlement plate array designs for sampling invasive ascidians. 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 Victoria and 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 10). Arrays are usually placed with floats to suspend plates about 1-2 metre below the surface and tied off 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 two months to allow biofouling to reach a size and maturity to enable effective taxonomic identification. Material scraped from settlement plates deployed for 6-8 weeks can alternatively be tested using DNA-based methods such as qPCR 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 ascidian 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.

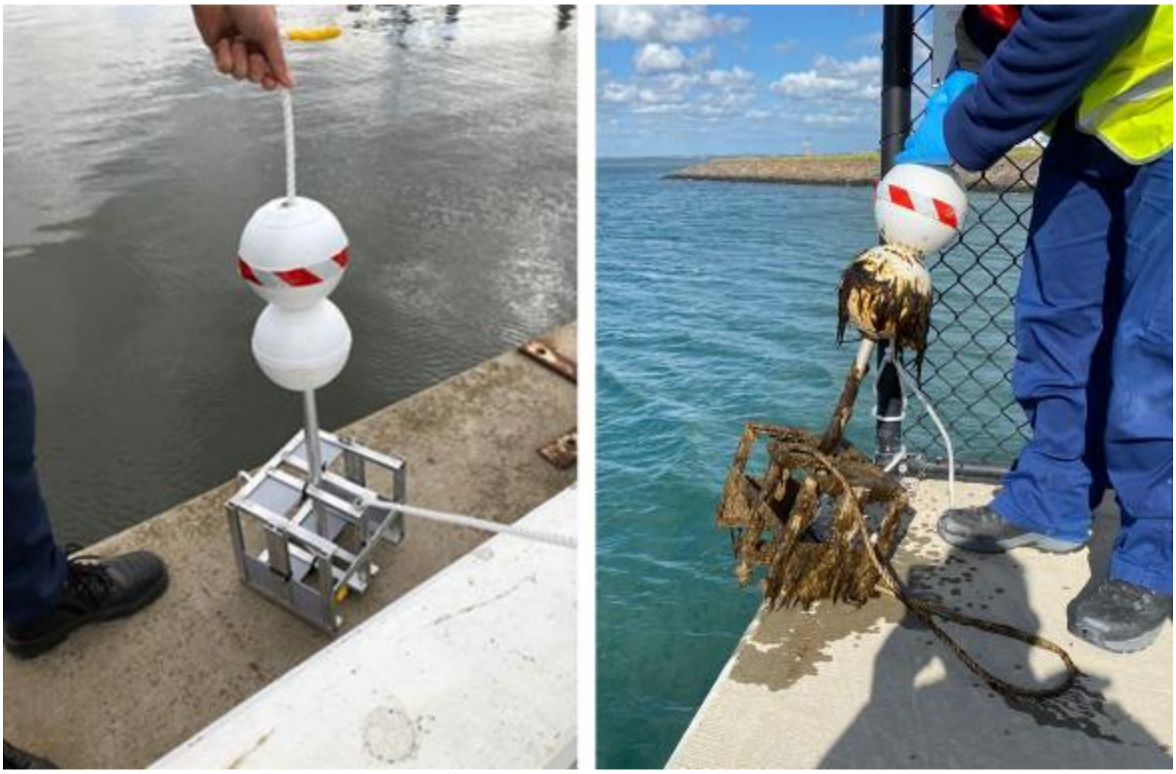
Experiments indicate that ascidians prefer rough plastics such as high-density polyethylene which can be scuffed to roughen the surface, and concrete, to substrates such as smooth plastics (e.g. polyvinyl chloride), granite, wood, and aluminium (Chase et al. 2016; Ma 2012). It is also recommended to submerge plates in seawater for two weeks prior to usage, as ascidians seem to avoid clean surfaces without biofilms (Chase et al. 2016; Deibel et al. 2014).

Since ascidian larvae are negatively phototaxic (Aldred & Clare 2014; Daley & Scavia 2008; Deibel et al. 2014), settlement plates may be colonised more often in shaded areas (e.g. underneath a jetty). Given the limited reproductive activity of many ascidians during winter (see Reproduction, life cycle, and growth), settlement panels are more likely to detect ascidians in summer and autumn months (Smale & Childs 2011). Sets of plates can be sequentially deployed to determine the timing of larval settlement (Deibel et al. 2014).

Deibel et al. (2014) recommend photographing plates *in situ* underwater, and then photographing plates under standard lighting conditions within a laboratory. Specimens can be removed for morphological or genetic analysis and plates can be examined using microscopy or image analysis to identify smaller zooids and colonies (Deibel et al. 2014).

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 10 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, water containers or water samplers to test for eDNA of target ascidian species. Many invasive ascidians will have planktonic larval stages or shed DNA or DNA-containing particles (e.g. gametes after spawning or broken fragments) into the water column, 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 ascidian 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. 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. 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,b; 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 species-specific probe-based 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 but will be dependent on the target 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 ascidians by-hand). Visual shore searches are commonly undertaken during both active and general surveillance activities.

Many invasive ascidians can occur in the intertidal zone, permitting shore-based surveillance and sample collection during low tides (Auker 2019; Tobias-Santos et al. 2024). During inspections of maritime areas like harbours and marinas, ascidians can often be spotted under complex structures such as jetties without diving. Pulling up submerged ropes and lines is also a reliable method to find ascidians (Coutts & Forrest 2005; Rius et al. 2008; Tobias-Santos et al. 2024). Beyond anthropogenic structures, ascidians generally prefer rocky shores. Large bivalves such as oysters or barnacle shells are also favoured settlement sites. Seasonal seaweeds such as Sargassum can also harbour a wide variety of ascidians.

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 ascidian species, then many searchers can be deployed, covering large areas. Like divers, searchers can take photographs or physically collect invasive ascidians for identification.

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 (i.e. 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 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.

Visual shore searches can be particularly valuable when done by citizen scientists for intertidal species. With some training citizen scientists may be able to contribute to delimitation surveillance to increase scope very cost effectively.

5 Containment, control, and eradication

Containment, control, and eradication may be attempted after the confirmed detection of an invasive ascidian. However, given the time for confirmation of identification, if there is reasonable likelihood that the suspected Ascidian is invasive, appropriate containment measures should be implemented to minimise risk of spread.

Management options usually include:

- Containment and control of the invasive ascidian to the infested areas and prevention of further spread; incurs ongoing costs and efforts, or,
- eradication of the invasive ascidian 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 ascidians, 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 ascidian. 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 the ascidian translocation from the infested waterway. This may include movement controls on potential vectors, public information campaigns, policies and practices for vessels 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 ascidian (see Section 5.3 for control methods).

5.2 Eradication

Eradication of any invasive ascidian requires complete elimination of the species from the infested area. Eradication programs of any invasive ascidian will have higher likelihood of success 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. In open coastal waters with moderate-to-high water exchange, emergency containment is likely to be limited to ascidians with limited adult and larval dispersal or those which reproduce by vegetative growth or budding from the edges of a colony. 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 have been no known successful eradication attempts on invasive ascidians globally, however attempts were made for *D. vexillum* in New Zealand and Australia.

Eradication is the preferred response option when:

- the ascidian 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 calculated to be cost-effective.

The biology and reproductive strategy of an invasive ascidian will influence the effectiveness of an eradication program. Due to spread by tides and currents, eradication is more challenging in 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 against 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 they have the opportunity 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 ascidian incursion and help define likelihood of eradication.

Table 4 Variables to describe the nature of an invasive ascidian incursion

Variable	Distribution level
Area currently infested	Very small (<100 m²) Small (100–1 000 m²) 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
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)

Variable	Distribution level
Water exchange in incursion area	Minimal Low High

Source*:* Modified from Crombie et al. (2007)

5.2.1 Infestations in open coastal environments

There are limited emergency eradication response options available for invasive ascidians in open coast environments, particularly on high energy coastlines or water >10 metres deep. Many treatment 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 dispersal of larvae (or asexual tissue fragments for colonial ascidians) if reproduction or budding/fragmentation is occurring and maintaining treatment conditions for enough time to be effective. For instance, the application of chemicals will require development of support structures or technologies that account for current and wave action effects. 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. Successful eradication usually combines a range of methods, some of which may be selected based on factors such as population distribution and density (Green & Grosholz 2020).

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 ascidian 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 draining, encapsulation, smothering, manual removal, and the application of chemical biocides. Timeliness is essential, because if the invasive ascidian has spawned and the larvae have settled, then control will be far more difficult. Repeat treatments 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). 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 the ability of monitoring and delimitation surveys to locate and treat all clusters of the ascidian population.

In areas where ascidians 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 ascidian species and delimitation survey results.

5.3 Methods for containment, control, and eradication

Methods and treatments that have been trialled for containment, control, and eradication of invasive ascidians are listed in this section. The methods used to treat invasive ascidians 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 details 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 ascidians can be divided into three generic treatment types:

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

The acceptability of control methods depends on their feasibility, effectiveness, cost, public support, and off-target effects.

The biology and ecology of invasive ascidians also needs to be considered when selecting appropriate control methods. The efficacy of the control method can be impacted by the ecology of the ascidian (i.e. controls for solitary and sexually reproducing species will likely be different to controls for colonial and asexually reproducing 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 ascidian populations are summarised in this section. 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 invasive ascidians or kill them *in situ*. Physical treatments are generally the most socially and environmentally acceptable way of removing invasive ascidians from a system. Physical treatments can be difficult to achieve in complex habitats often inhabited by ascidians, such as reefs, 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 seabed or on artificial structures, such as a hull surfaces in a contained marina.

Desiccation and encapsulation are arguably the most effective physical treatments for invasive ascidians. When it is possible to remove vessels or objects from water, a combination of prolonged air-drying, exposure to direct sunlight, and manual removal is highly effective. Hauling out vessels and large structures can be costly and time consuming and is not always a feasible option.

When fouled objects or structures cannot be removed from water, or if seafloor substrates are contaminated, encapsulation and smothering can also be highly effective. Under both approaches, great care must be taken to avoid dislodging ascidians or fragmenting colonial taxa, as this can facilitate spread. Impermeable plastic wrapping and commercial devices can be used to encapsulate small vessels and previous response efforts have tested suction methods to collect debris. Given the risks associated with dislodgment and fragmentation, it is generally recommended to complement these physical approaches with chemical treatments such as lime or acetic acid. Alternative physical treatments such as aeration and ultrasound are also discussed in this section.

5.3.1.1 Desiccation and water level manipulation

Desiccation involves the removal of ascidians from the water. Sunlight in combination with desiccation is extremely effective as a general disinfectant. Desiccation via air-drying can be an effective treatment to eliminate invasive ascidians, but some solitary species can survive days or weeks of air exposure depending on air temperature and humidity, as well as exposure to direct sunlight. Desiccation is only practical where removal of fouled surfaces from the water for an extended period is possible.

The recommended length of time required for effective desiccation of colonial ascidians is 48 hours of continuous air-exposure (Deibel et al. 2014), but at least two weeks of air-drying is required for desiccation-resistant taxa such as *Styela* (Hillock & Costello 2013). Treatment times can be significantly reduced by combining air-drying with chemical treatments and manual removal (Deibel et al. 2014; Inglis et al. 2013). Experiments involving adult populations of *Ciona* spp. found that air exposure caused 100% mortality within 24 hours, with most individuals dying within two hours (Hopkins et al. 2016). The presence of sea spray did not affect the survival of *Ciona* adults, while larvae remained viable for up to eight hours (Hopkins et al. 2016). Another experiment indicates that botryllid ascidians can survive at least six hours of air exposure, whereas species of *Molgula* survived 24 hours of exposure with low rates of mortality (Kauano et al. 2016).

Lowering water levels in a water body can cause mortality of submerged organisms through desiccation. Water level manipulation may be a suitable treatment method for invasive ascidians. 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.

At least two weeks of continuous air exposure with an air temperature of at least 10°C is recommended to kill *S. clava* (Hillock & Costello 2013). Overall, desiccation is not considered a viable method for the destruction of *Styela* spp., although experiments indicate that desiccation to remove fouling ascidians is less harmful towards aquaculture mussels than chemical treatments using acetic acid (LeBlanc et al. 2007).

Like many stolidobranch ascidians, it is hypothesised that *Styela* spp. individuals can survive air exposure for extended periods by closing their siphons, most likely up to five or six days, but potentially as long as 11 days (Coutts & Forrest 2005; Darbyson et al. 2009; Hillock & Costello 2013; Kauano et al. 2016; Sims 1984). Experiments have shown that mortality remains low between 24 and 48 hours of exposure (Darbyson et al. 2009; Hillock & Costello 2013; Kauano et al. 2016). Likewise, two rounds of air exposure for six hours, 15 days apart, caused 27% mortality in *S. plicata* (Santos et al. 2023). Experiments with *S. clava* indicate that the efficacy of desiccation is affected by ambient air temperature and humidity, as well as exposure to direct sunlight (Coutts & Forrest 2005; Darbyson et al. 2009; Hillock & Costello 2013).

5.3.1.2 Wrapping and encapsulation

Wrapping or encapsulating submerged structures or vessels in an impermeable plastic layer is a highly effective treatment to eradicate ascidian biofouling. 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 substantial rise in toxic ammonia and sulphide compounds, quickly leading to ascidian mortality (Coutts & Forrest 2005; Keanly & Robinson 2020). Invasive ascidians will be deprived of light and food, with continued respiration and decomposition of organisms within the barrier depleting oxygen to lethal levels. A secondary advantage of encapsulation is that it can reduce the spread of ascidian larvae. For the greatest impact, the targeted ascidians needs to be enclosed during winter months before the reproductive season occurs (Deibel et al. 2014).

The effectiveness of wrapping is improved by the following:

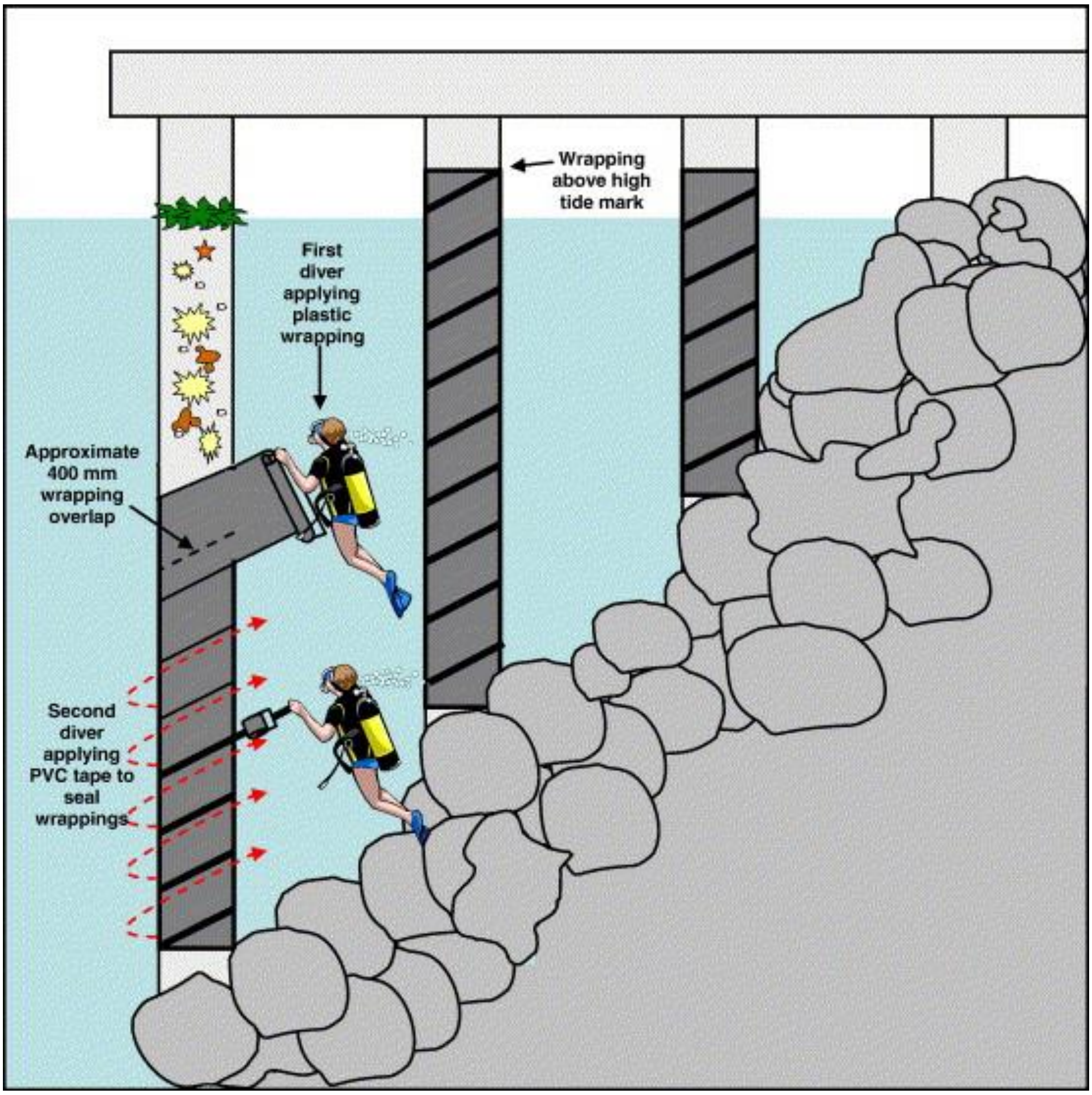
- smothering material is applied continuously without gaps, breaks, or tears to prevent escape of fragments or larvae or ingress of clean water
- use in sheltered environments with low currents because strong currents can make deploying the wrap difficult and increase the risk of tearing
- addition of 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. Given the anoxic and toxic conditions created by encapsulation, this is not a suitable treatment for ascidian epibiosis on aquaculture or protected wild species (Muñoz & McDonald 2014).

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 seven 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; Mitchell 2007) 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 8**Error! Reference source not found.Error! Reference source not found.**; 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 8 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 ascidians are not dislodged or fragmented when the wrap is deployed (particularly for colonial taxa). Generally, the wrap must remain in place for at least seven days if no biocide is used to achieve the desired effect (Coutts & Forrest 2005; Inglis et al. 2013). Temperature most likely influences mortality rates. For instance, Coutts & Forrest (2005) predicted that complete mortality for *S. clava* would take longer than seven days at cooler temperatures, and 100% mortality for encapsulated *Ciona* spp. took ten days in temperate New Zealand waters (Atalah et al. 2016). Complete mortality for *C. robusta* was achieved within 24 hours in warmer South African waters (Keanly & Robinson 2020).

Wrappings can themselves be colonised by invasive ascidians (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.

5.3.1.3 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 of invasive ascidians on the seabed. Smothering has:

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

The material used to smother the surface must be continuous, without gaps or breaks in material to avoid escape of fragments or larvae. For an incursion response in New Zealand, divers emptied polypropylene woven bags (840 x 460 mm) to smother *D. vexillum* under 100 mm of uncontaminated dredge spoil (Coutts 2006). This spoil smothering was highly effective on a gentle seabed slope but it was less effective on a steeper slope with rip-rap (Coutts & Forrest 2007). The same response team discovered that sheets of high-grade geotextile fabric (50 × 8 m), which have a pore size small enough to contain ascidian larvae, also highly successfully smothered *D. vexillum*, and they hypothesised that sheet smothering would have been more effective than spoil over the steeper rip-rap slope (Coutts & Forrest 2007).

5.3.1.4 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). Manual removal of invasive ascidians can be achieved in water by divers, or via removal from man-made structures on land (e.g. vessels and aquaculture equipment) and the intertidal zone (intertidal reefs) (Pleus et al. 2008). 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.

The advantages of manual removal are selectivity for the target ascidian and limited damage to non-target species (Pleus et al. 2008). 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 so 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).

Although they are labour intensive and time consuming, manual methods such as handpicking, brushing, and scraping can be effective for the removal of solitary ascidians (Muñoz & McDonald

2014; Pleus et al. 2008). However, these methods are ineffective for colonial species as they can cause fragmentation and potentially spread of invasive taxa (Muñoz & McDonald 2014; Switzer et al. 2011). Manual removal can be complemented with other methods such as chemical treatments and encapsulation (Muñoz & McDonald 2014; Switzer et al. 2011).

5.3.1.5 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. Care must be taken during mechanical removal because colonial ascidians may be capable of regenerating from fragments. Mechanical removal will increase the area capable of being treated compared to manual removal. Mechanical brushes and underwater brush cart systems can be more effective than manual methods for defouling large surface areas (Morrisey & Woods 2015), but they are unsuitable for colonial species unless all fragments are effectively contained. Rotating brush systems combined with suction have been tested for the removal invasive ascidians (see Section 5.3.1.6 below).

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).

5.3.1.6 Underwater vacuum, suction, and filtration 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 vacuum is best suited to infrastructure or sites where substrates are primarily sandy. The Marine pest response manual lists suction as a mixed success tool for the removal of *D. vexillum*. Multiple, repeated treatments with suction devices may be a potential solution to increase removal rates.

Underwater vacuuming and filtering to remove *D. vexillum* in New Zealand was deemed expensive, ineffective at removing colonies from the seabed, and facilitated *D. vexillum* spread considerably while the trial was conducted (Coutts & Forrest 2007; Morrisey & Woods 2015). Diver-operated rotating brush systems coupled with a suction system for collecting debris (i.e. combined manual and suction removal) are effective for removing low-to-moderate levels of fouling (including colonial ascidians), but their performance may be poor at higher levels (Hopkins et al. 2008). While device may capture >95% of removed material, the approach is considered inappropriate because it is easy for divers to inadvertently detach fouling organisms while manipulating the devices or miss patches due to human error or the inaccessibility of the brushes into niches (Hopkins et al. 2008). Inglis et al. (2013) assessed underwater suction devices and rotating brushes, concluding suction methods were effective for removing large, soft-bodied ascidians but otherwise ineffective, and that rotating brushes were unsuitable for niche areas and had the potential to damage biocidal coatings (Inglis et al. 2013).

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.

5.3.1.7 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. High-pressure water blasting can be an effective tool for removing invasive ascidians, but it is time consuming and labour intensive, and it can only be used on surfaces such as concrete and metal that can withstand the pressure without disintegrating or releasing pollutants (e.g. creosote) (Pleus et al. 2008). High-pressure water blasting can also fragment colonial species, increasing their potential to disperse and persist (Aldred & Clare 2014).

High-pressure water blasting (~700 psi) is effective at removing *B. schlosseri* and *B. violaceus* colonising mussel aquaculture equipment (Arens et al. 2011). Paetzold et al. (2012) found similar results for the same ascidian species, although they emphasised that frequent high-pressure water blasting is not viable given the cost of the treatment and the risk of spreading colonial ascidians via fragmentation. Notably, fragments of *B. schlosseri* have been demonstrated to be viable at least 18 days after high-pressure water treatment (Paetzold & Davidson 2010). 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.

5.3.1.8 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 ascidians has not been specifically tested.

5.3.1.9 Thermal treatment

The efficacy of any thermal treatment is dependent on the susceptibility of the target ascidian's life stages and the ability to maintain the required temperature to achieve mortality. Thermal treatments have been tested quite widely on other common biofouling organisms (Morrisey & Woods 2015), but they have not yet been tested significantly upon ascidians. 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, and soft-bodied organisms (Cvetkovic et al. 2015). Complex topographies or heavy fouling 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.

Ropes fouled with *C. intestinalis* and *S. clava* dipped in seawater or citric and acetic acid solutions heated to 40 or 50°C for ≤1 minute results in 100% mortality (Sievers et al. 2019). Chemical treatments with increased temperatures are more effective than ambient temperature chemical treatments (Sievers et al. 2019).

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).

Coutts & Forrest (2007) trialled blowtorches and hot water blasting at a pilot scale to remove fouling *D. vexillum*, but these methods were not considered economically feasible given the large area inhabited by the invasive ascidian. Similar hot water treatments were particularly ineffective for removing unidentified ascidians on uneven, irregular surfaces (Blakemore & Forrest 2007).

5.3.1.10 Benthic sampling and removal

While many invasive ascidians are more likely to grow on walls and on suspended and floating structures, sled tows can be an effective method to sample epibenthic assemblages over large areas (Photo 11).

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 ascidians attached to hard surfaces. Sled tows can cause fragmentation and potential spread of invasive colonial ascidians. 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 11 Epibenthic sled and use of sled underwater

Source*:* Chris Woods, Earth Sciences New Zealand

5.3.2 Chemical treatment

The dynamic nature of marine environments means that any biocides or chemical agents, such as chlorine, salt, herbicides, 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 large 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. Anti-foulants are an important tool in reducing the spread of marine pests, see Section 3.3.1 for more information.

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 chemical)
- 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 ascidians 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.

Lime and acetic acid, two highly caustic chemicals, are generally considered to be most effective chemical treatments for invasive ascidians (Pleus et al. 2008), although results for reproductive inhibitors are promising and warrant practical field trials. While current research is limited, acetic acid seems to be the most harmful treatment for non-target organisms, which limits its application in aquaculture settings. Many studies have also investigated chlorine, osmotic and citric acid treatments with mixed results.

These chemical treatments can effectively complement physical approaches such as encapsulation, smothering, and desiccation. The pH changes associated with strong acids and alkali are likely to affect anti-fouling paints however (Morrisey & Woods 2015).

Silicic acid (sodium metasilicate) and caustic lye/soda (sodium hydroxide) have moderate results for the removal of *D. vexillum* (Denny 2008). Ammonium sulphate solution (with added acid- and akali-stable surfactants) is identified as potential control agent for *E. elongatum* (Page et al. 2011). However, an experiment investigating the interacting effects of copper paint and a commercial fertiliser mix (including ammonium, potassium sulphate and sulphur) found that fertiliser increased recruitment and decreased mortality of didemnid ascidians (Lawes et al. 2016). Formalin and detergents have been tested to remove invasive ascidians, but no results have been published (LeBlanc et al. 2007; Muñoz & McDonald 2014). Chemicals that target cellulose or cellulose-like compounds are likely to be highly effective against ascidians (Pleus et al. 2008).

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 ascidians is likely to differ from that specified on the label. In these cases, permits need to be sought from APVMA to use chemicals for ascidian control. 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 ascidians, 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 Lime

Active lime (calcium hydroxide; hydrated lime) is an economical form of chemical treatment because it is produced in large quantities for commercial purposes, is relatively inexpensive, and only small quantities of active forms of lime are needed to treat most benthic organisms.

Active lime is a generally effective chemical treatment for invasive ascidians via dipping or spraying (Lambert & Lambert 2023; Willis & Woods 2011). Eight minutes of exposure to 4% active lime causes high mortality in *C. intestinalis* (Carver et al. 2003). However, shorter exposure periods (< 1 minute) within the same concentration range results in low mortality (Denny 2008). For longer treatments, Piola et al. (2010) found that exposure to 5% active lime for six hours, or exposure to 10–20% active lime for at least 30 minutes, is sufficient to eliminate a significant proportion of *C. intestinalis, B. leachii* and *B. schlosseri*. Active lime was also tested as a treatment for *E. elongatum* (Morrisey et al. 2009; Page et al. 2011).

The environmental concerns associated with lime are poorly understood. Some experiments indicate that active lime is ineffective for removing other fouling organisms (Piola et al. 2010). In aquaculture applications, brief exposures to 3–5% active lime either have a mixed effect or do not significantly affect oyster survival, growth or condition (Lambert & Lambert 2023; Willis & Woods 2011). Active lime is also considered to be an effective treatment for *Molgula* sp. without causing significant harm to mussel spat (Locke et al. 2009).

Powdered active lime is considered more practical than pellets of quicklime (calcium oxide) (Locke et al. 2009). Active lime can be somewhat impractical to apply, because active lime powder is insoluble and undissolved lime can clog sprayer nozzles (Piola et al. 2010). Direct contact with active lime is required for a significant effect to be achieved. Appropriate PPE is always required due to the caustic effect of active lime.

5.3.2.3 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 et al. 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 et al. 2007). When treating aquaculture stock, it is important to understand the minimum time required to remove the marine pest and minimise stock mortality.

Acetic acid is a low-cost treatment for invasive ascidians that can be applied via spraying and dipping (immersion) (Carver et al. 2003; Locke et al. 2009; Muñoz & McDonald 2014; Page et al. 2011). Exposure to 5% acetic acid for 30 minutes is generally sufficient to cause high ascidian mortality.

While acetic acid often matches or outperforms alternative chemical treatments, evidence indicates that it is more harmful to non-target wild and aquaculture organisms (Cahill et al. 2013).

The concentration of acetic acid to kill invasive ascidians should be considered prior to application. Reviews have recommended 5% acetic acid (Morrisey & Woods 2015; Muñoz & McDonald 2014), and two minutes of exposure to 10% acetic acid to eliminate the colonial species *D. vexillum* (McCann et al. 2013), but up to ten minutes of exposure to 1–4% acetic acid caused high but not 100% mortality (Denny 2008). Likewise, Roche et al. (2015) reported 81% mortality for *D. vexillum* after 30 minutes of exposure to 5% acetic acid, and other studies have reported similar results (Piola et al. 2010; Rolheiser et al. 2012). It has been hypothesised that the survival of a small proportion of colonies might be related to the acidic surface of didemnid ascidians, which is only a slightly higher pH than acetic acid (Denny 2008). Previous research indicates that 5% acetic acid is also sufficient to eliminate other colonial species such as *B. schlosseri*, *B. leachii* and *E. elongatum* (Forrest et al. 2007; Page et al. 2011).

Similar results are reported for solitary ascidians. Coutts & Forrest (2005) reported that 10 minutes of exposure to 1% acetic acid was sufficient to cause 100% mortality in *S. clava*, with 5% acid causing complete mortality within 1 minute (Coutts & Forrest 2005). High mortality rates for *S. clava* and *S. plicata* have also been demonstrated for ≤2-minute immersion in 4 or 5% acetic acid (Santos et al. 2023). Similar results have been reported for *C. intestinalis*, with 1 minute of exposure to 2–5% acetic acid causing high or complete mortality (Atalah et al. 2016; Carver et al. 2003; Sievers et al. 2019).

Repeated spraying can mitigate incomplete mortality and significantly reduce exposure times. Exposing *D. vexillum* to one-minute spray intervals of 5% acetic acid achieves almost 100% mortality faster than a single-spray treatments (Piola et al. 2010). Acetic acid can be paired effectively with encapsulation (Atalah et al. 2016; Coutts & Forrest 2005; Forrest et al. 2007; Muñoz & McDonald 2014) and desiccation (Carver et al. 2003; Forrest et al. 2007; Page et al. 2011). Combining acetic acid with heat treatment (40 or 50°C) can also increase the effectiveness of lower concentrations and shorter immersion times (Sievers et al. 2019).

While acetic acid is an effective treatment for invasive ascidians, it also negatively affects the survival of aquaculture species and other non-target organisms (Muñoz & McDonald 2014). Rolheiser et al. (2012) reported 100% oyster mortality in experimental treatments lasting five and ten minutes, with lower concentrations and shorted exposure periods causing ~40% mortality. Acetic acid treatments can also cause high mortality rates in mussels (LeBlanc et al. 2007; Santos et al. 2023), and hamper their reattachment capability (Forrest et al. 2007). Some evidence indicates that there are no long-term consequences for the fitness of surviving shellfish (LeBlanc et al. 2007), but more research is needed (Atalah et al. 2016; Santos et al. 2023). In comparison, lime is considered to have a less significant impact on shellfish survival (Rolheiser et al. 2012). Prolonged, 14-day exposure to high concentrations of acetic acid also causes 100% mortality in fish and shrimp (Locke et al. 2009).

Some authors have reported that acetic acid can be challenging to spray evenly (Locke et al. 2009). Low concentrations of acetic acid (1–5%) are equivalent to vinegar and easy to handle and dispose of with appropriate PPE (Forrest et al. 2007). However, pure acetic acid freezes and solidifies below 17°C, which makes the handling and storage of higher concentrations more challenging (Coutts & Forrest 2005). Users should remember that acetic acid is highly corrosive and produces strong odours, which is why some researchers consider chlorine to be safer for removing ascidian fouling over large areas (Morrisey & Woods 2015; Roche et al. 2015).

5.3.2.4 Citric acid

Citric acid may be a suitable alternative chemical treatment to acetic acid, although further research is required.

Citric acid has similar or slightly reduced effects for the removal of *S. clava* and *C. intestinalis* compared to acetic acid, but causes slightly higher mussel and oyster mortality compared to acetic acid (Sievers et al. 2019). Further investigation is required for the application of citric acid to remove invasive ascidians in aquaculture applications (Sievers et al. 2019).

5.3.2.5 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.

Research on the efficacy of chlorine on invasive ascidians shows varying results. Despite this uncertainty, chlorine is a straightforward tool to augment encapsulation and smothering treatments (Coutts & Forrest 2005; Coutts & Forrest 2007; Daley & Scavia 2008; Roche et al. 2015). Chlorine is also considered a safer option compared to acetic acid for treating ascidian fouling over large areas (Morrisey & Woods 2015; Roche et al. 2015).

At least ten hours of exposure to >200 g/m^3 sodium hypochlorite solution is required for 100% mortality of *D. vexillum* and *S. clava*, with free available chlorine maintained at >20 g/m^3 during this time (Coutts & Forrest 2005; Coutts & Forrest 2007). 100% mortality for *D. vexillum* may be achieved after ten minutes of immersion in 1% bleach (McCann et al. 2013). However, using the same target species and concentration of bleach, Roche et al. (2015) achieved 80% mortality after two weeks.. Immersion in 0.5% bleach for 20 minutes may be sufficient to entirely eliminate *D. vexillum* transfer on mussel spat (Denny 2008).

Variation in reported chlorine efficacy is likely a reflection of the variation among ascidian taxa as well as differences in the design, scale, exposure and ambient conditions of each experiment. For example, spraying and immersion with 60 ppm bleach for 20 minutes had no effect on *C. intestinalis* survival (Carver et al. 2003), and immersion in 1.5% bleach for 1.5 minutes was an ineffective treatment against *S. plicata* (Santos et al. 2023). These findings suggest that bleach may be a less effective treatment for solitary ascidians.

Chlorine can negatively affect non-target aquaculture organisms, although more research is required. Denny (2008) associated only 6% mussel spat mortality with 20 minutes immersion in 0.5% bleach, but Santos et al. (2023) reported 55.6% adult mussel mortality after 1.5 mins exposure to the same concentration of bleach. Differences may be caused by the species of mussel, the size and sexual maturity individuals, or acclimation temperatures (Santos et al. 2023).

5.3.2.6 Osmotic treatment

Osmotic treatment, i.e., the manipulation of salinity levels, 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. Treatment 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, which 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 ascidians will depend on their ability to withstand prolonged exposure to an altered regime.

Osmotic treatments using freshwater and brine are generally more effective with prolonged exposure periods, although they are generally perceived to be less effective than other chemical treatments such as lime and acetic acid. It should be noted that the results of osmotic studies frequently contradict one another, which suggests that conditions such as water temperature probably impact results or that ascidian taxa vary in susceptibility.

Continuous immersion of fouled structures in freshwater should result in 100% mortality for solitary *Styela* within 24 hours, but salt leaching from immersed objects may enhance ascidian survival (Coutts & Forrest 2005). It has also been reported that *S. plicata* can withstand 'periods' (almost certainly <24 hours) of exposure to ≥8 ppt, as the species can close its siphons (Forrest 2013). Aquaculture experiments with 14-day exposure to reduced seawater salinities (30–37 ppt) prevented sexual reproduction in *Ciona oblonga*, with lower salinities (11–20 ppt) causing necrosis and fragmentation (Majnarić et al. 2022).

In closed experimental aquaria, 100% mortality of *D. vexillum* colonies is achieved with continuous immersion in freshwater for four hours or at least 24 hours in brine solution (62 ppt), and 98% mortality in brine immersion for four hours (McCann et al. 2013). Likewise, Denny (2008) found that ten minutes of freshwater immersion can cause 87% mortality in *D. vexillum.* However, in contrast, Rolheiser et al. (2012) found that brine and freshwater treatments, tested with a range of exposure times between 30 minutes and 10 hours, were almost entirely ineffective for removing *D. vexillum* from fouled oysters. All three studies used ambient temperature water, ranging between 12.0 and 16.9°C (Rolheiser et al. 2012; Switzer et al. 2011).

Brief osmotic treatments seem to be less effective. Exposing the solitary species *C. intestinalis* to brine for eight minutes or freshwater for 1 minute only resulted in 20% and 10% mortality respectively (Carver et al. 2003). Likewise, experiments using *S. plicata* found only 27% mortality from freshwater immersion (30 minutes, repeated 15 days later for one hour) (Santos et al. 2023).

5.3.2.7 Combined chemical and physical treatment

Speed of effectiveness of wrapping, encapsulation, and smothering can be accelerated with the addition of chemical treatments such as acetic acid and chlorine (Atalah et al. 2016; Coutts & Forrest 2005; Coutts & Forrest 2007; Keanly & Robinson 2020; Muñoz & McDonald 2014; Roche et al. 2015).

For wrapped structures, it is recommended to directly add a biocide to the enclosed water and not to use pumping as it is inefficient and risks breaking seals within a barrier (Coutts & Forrest 2005). However, commercially available in-water encapsulation devices for vessels can be effectively fitted with pumps to administer chemical treatments (Keanly & Robinson 2020; Roche et al. 2015).

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 because 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).

Biological control species are not recommended as a viable method for the control of invasive ascidians (Muñoz & McDonald 2014; Pleus et al. 2008). Ascidians do not seem to be preferred prey (Carver et al. 2006; Epelbaum et al. 2009) and asexual, vegetative growth in colonial ascidians usually outpaces predator consumption (Atalah et al. 2014). Didemnid ascidians such as *D. vexillum* also have a variety of adaptations that deter predation and increase their chance of survival (see Trophic ecology above).

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 ascidians. 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 methods that can assist with decontamination and destruction of invasive ascidians in addition to the above AQUAVETPLAN manuals.

6.1 Decontamination

Decontamination is the cleaning or treatment of material used to remove invasive ascidians or render ascidians non-viable, including their propagules and any parasite and pathogen that can be associated with the marine pest species (Williams & Grosholz 2008; 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 ascidians 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:

- form of ascidian (solitary or colonial) 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 an invasive ascidian from disinfecting agents. Wash water may still contain viable gametes, larvae, or tissue fragments 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 ascidian 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 a 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 an invasive ascidian (see Section 5.3.1.1). In aquaculture contexts, tolerance to desiccation should be considered for both the ascidian and the farmed aquaculture stock prior to removal from the water.

Invasive ascidians 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 ascidian from the water and destruction should be as short as practically possible. This will minimise the organism's stress and prevent release of the viable propagules and fragments.

Where invasive ascidians are removed and destroyed elsewhere, the site used for destruction should be contained to prevent release of the ascidian, viable propagules and fragments, or pathogens and parasites. Ideally the destruction site should be close to the area from which ascidians 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 ascidians, 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 form of ascidian (solitary or colonial) to be destroyed
- how the ascidian will be contained until destruction
- any pathogens or parasites carried by the ascidian that will also need to be destroyed
- facilities and equipment available for destruction method
- appropriate destruction methods (see Section 4 and 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 invasive ascidian 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 ascidian 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 relevant ascidians manual

Appendix A provides a list of ascidian species relevant for this manual. Most species listed in this appendix are cited in the manual or frequently referred to in cited literature. This appendix does not cover every potential or introduced invasive ascidian to Australia. Not all listed species are considered invasive taxa to Australia and many have not yet been detected in Australia. The species are separated by family, then listed in alphabetical order for ease of use. Additional photos (beyond the main text photos) are provided for many taxa. Cryptogenic taxa are included because the geographic distributions of many ascidians remain uncertain, and both regionally native and cryptogenic species can still be invasive and a significant risk for Australia. Non-invasive taxa to Australia are sometimes discussed as they provide relevant information regarding ascidian biology and control methods. In addition, cryptogenic or native ascidians may be invasive overseas and are referred to for context for when the manual refers to overseas biosecurity responses. Hyperlinks to the species profile on the National Introduced Marine Pest Information System have been provided where available.

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

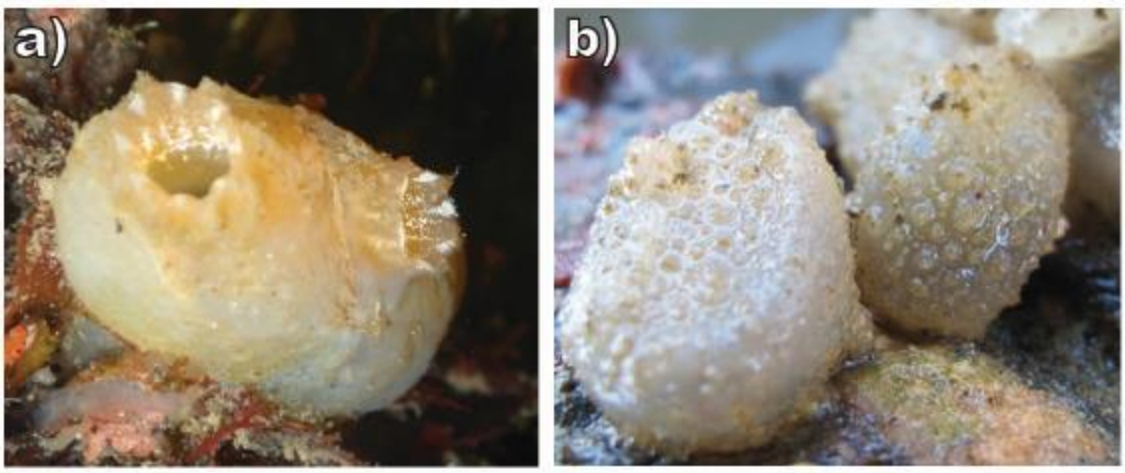
Ascidiidae

All Ascidiidae species are solitary with sexual reproduction.

Table 5 Ascidiidae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Ascidiella aspersa (Müller, 1776)	Established	Non-native	103718	Photo 12a,b
Ascidia sydneiensis Stimpson, 1855	Present	Cryptogenic	215940	n/a
Phallusia nigra Savigny, 1816	Not detected	Non-native	103725	n/a

Photo 12 Ascidiidae species



Photos of ascidiid ascidians. a, b) *Ascidiella aspersa*. Sources: a) Jean-Paul Boerekamps.; b) D. Gordon, Earth Sciences New Zealand.

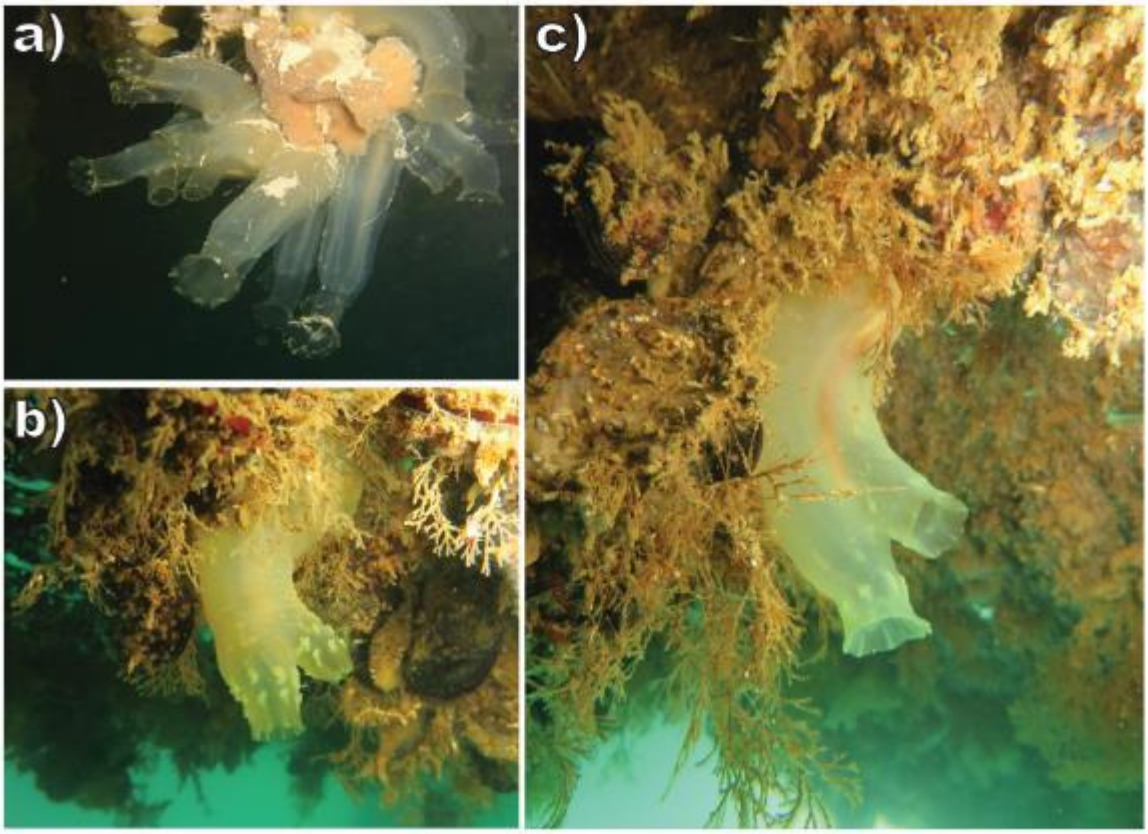
Cionidae

All Cionidae species are solitary with sexual reproduction.

Table 6 Cionidae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Ciona intestinalis (Linnaeus, 1767)	Established	Cryptogenic	103732	Photo 6e, Photo 13a
Ciona robusta Hoshino & Tokioka, 1967	Not detected	Non-native	252565	Photo 13b
Ciona savignyi Herdman, 1882	Not detected	Non-native	250292	Photo 6d, Photo 13c

Photo 13 Cionidae species



Photos of cionid ascidians. a) *Ciona intestinalis* from Lyttelton Harbour, New Zealand; b) *C. robusta* in Nelson harbour, New Zealand; c) *C. savignyi* from Whangārei Harbour, New Zealand. Source*:* a – c) C. Woods, Earth Sciences New Zealand

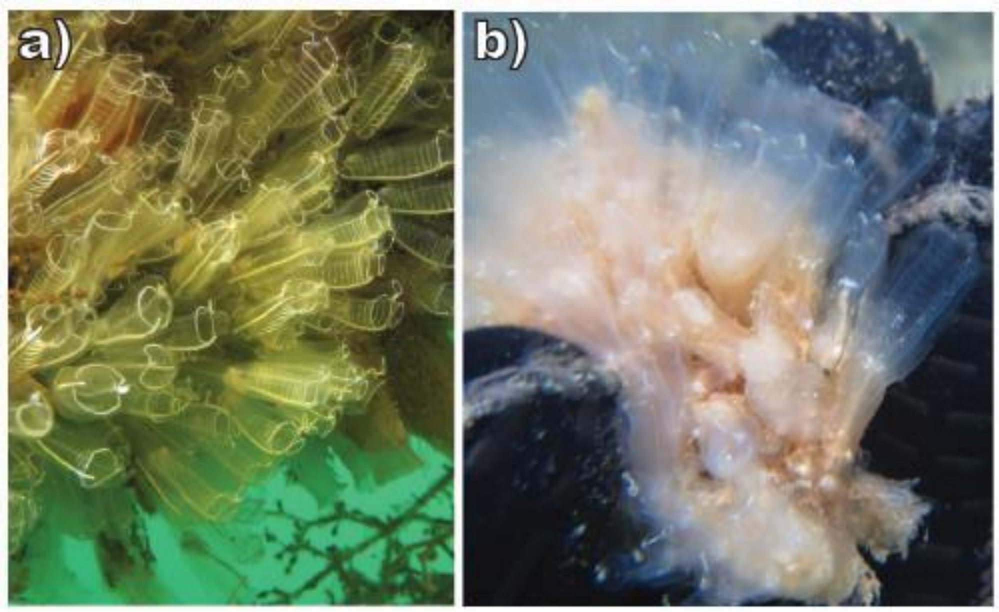
Clavelinidae

All Clavelinidae species are colonial with sexual and asexual reproduction.

Table 7 Clavelinidae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Clavelina lepadiformis (Müller, 1776)	Established	Non-native	103552	Photo 3a,b, Photo 5d, Photo 6d,f, Photo 14a
Clavelina oblonga Herdman, 1880	Not detected	Non-native	103554	Photo 14b
Pycnoclavella kottae Millar, 1960	Not detected	Non-native	266050	Photo 1d

Photo 14 Clavelinidae species



Photos of clavelinid ascidians. a) *Clavelina lepadiformis* in Tauranga Harbour, New Zealand; b) *Clavelina oblonga* from Great Barrier Island, New Zealand. Sources*:* a) C. Woods, Earth Sciences New Zealand; b) Samantha Happy, Auckland Council

Corellidae

All Corellidae species are solitary with sexual reproduction.

Table 8 Corellidae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Corella inflata Huntsman, 1912	Not detected	Non-native	250375	n/a
Corella eumyota Traustedt, 1882	Detected	Non-native	173223	Photo 15a

Photo 15 Corellidae species

Photo of corellid ascidians*:* a) *Corella eumyota* from Wellington Harbour, New Zealand. Source: C. Woods, Earth Sciences New Zealand

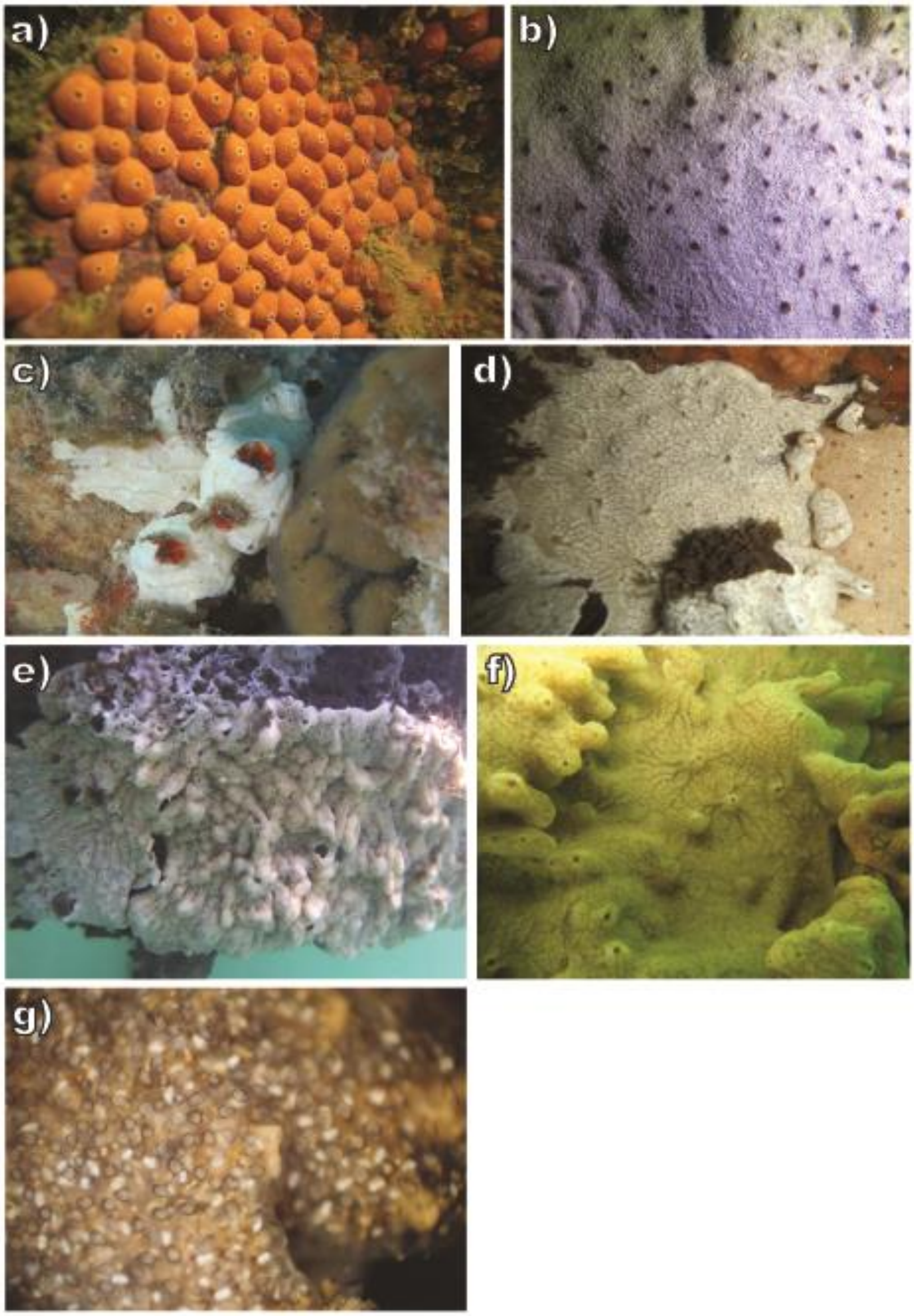
Didemnidae

All Didemnidae species are colonial with sexual and asexual reproduction. The genus *Didemnum* is especially speciose, and *D. perlucidum* and *D. vexillum* are high priority invasive species in Australia.

Table 9 Didemnidae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Didemnum albopunctatum Sluiter, 1909	Present	Cryptogenic	250466	n/a
Didemnum densum (Nott, 1892)	Not detected	Non-native	250494	Photo 16a
Didemnum elongatum Sluiter, 1909	Present	Native	250501	n/a
Didemnum grande (Herdman, 1886)	Present	Native	250516	Photo 16b
Didemnum lacertosum Monniot F., 1995	Present	Native	250532	n/a
Didemnum incanum (Herdman, 1899)	Present	Native	250524	Photo 16c
Didemnum molle (Herdman, 1899)	Present	Native	212513	Photo 16d
Didemnum moseleyi (Herdman, 1886)	Present	Native	250558	n/a
Didemnum multispirale Kott, 2001	Present	Native	250559	n/a
Didemnum nekozita Tokioka, 1967	Present	Native	250562	n/a
Didemnum ossium Kott, 2001	Present	Native	250567	n/a
Didemnum patulum (Herdman, 1899)	Present	Native	250576	Photo 16e
Didemnum perlucidum Monniot F., 1983	Established	Non-native	212506	Photo 6b,c, Photo 9d, Photo 16f
Didemnum psammatodes (Sluiter, 1895)	Non-detected	Non-native	252853	Photo 6c
Didemnum roberti Michaelsen, 1930	Present	Native	250588	n/a
Didemnum vexillum Kott, 2002	Established	Non-native	250126	Photo 3d, Photo 4c, Photo 7d, Photo 9a-d, Photo 16g
Diplosoma listerianum (Milne Edwards, 1841)	Present	Cryptogenic	103579	Photo 6d, Photo 7b, Photo 16h

Photo 16 Didemnidae species



Photos of didemnid ascidians. a) *Didemnum densum* on Waiheke Island, New Zealand; b) *D. grande*; c) *D. incanum*; d) *D. patulum*; e) *D. perlucidum*; f) *D. vexillum* in Shakespeare Bay, Picton, New Zealand; g) *Diplosoma listerianum* from Wainui, New Zealand. Sources: a) Shaun Lee; b, c, d, e) Matt Hewitt, DPIRD WA; f, g) Chris Woods, Earth Sciences New Zealand.

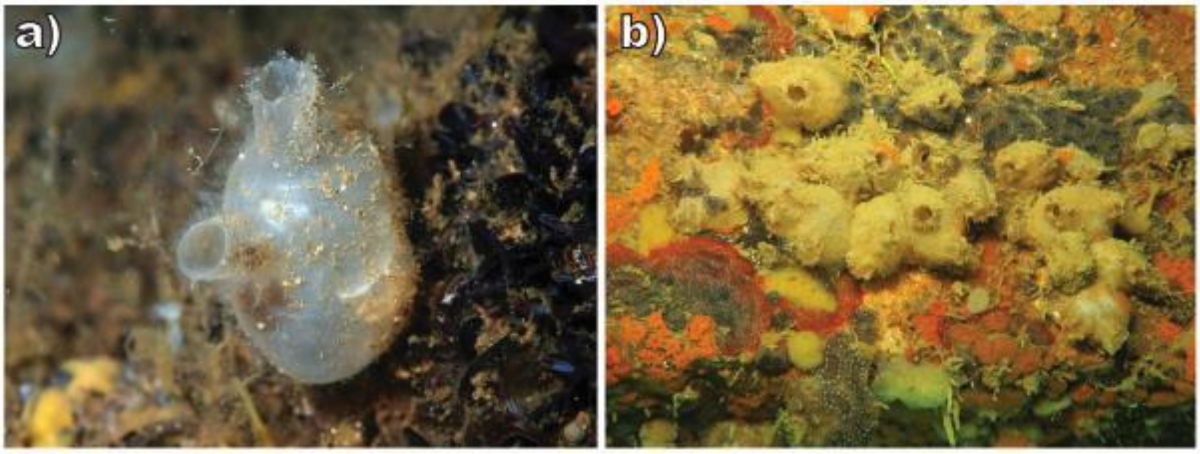
Molgulidae

All Molgulidae species are solitary with sexual reproduction.

Table 10 Molgulidae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Molgula manhattensis (De Kay, 1843)	Established	Non-native	103788	Photo 17a
Molgula mortenseni (Michaelsen, 1922)	Not detected	Non-native	250900	Photo 17b

Photo 17 Molgulidae species



Photos of molgulid ascidians; a) *Molgula manhattensis* on a jetty in Port-de-Bouc, France; b) *Molgula mortenseni* in Havelock, Marlborough, New Zealand. Source*:* a) Sylvain Le Bris; b) C. Woods, Earth Sciences New Zealand

Perophoridae

All Perophoridae species are colonial with sexual and asexual reproduction.

Table 11 Perophoridae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Perophora japonica Oka, 1927	Not detected	Non-native	103758	

Polycitoridae

All Polycitoridae species are colonial with sexual and asexual reproduction.

Table 12 Polycitoridae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Eudistoma elongatum (Herdman, 1886)	Present	Native	250210	Photo 3c, Photo 5c, Photo 7a, Photo 18 Polycitorida e speciesa
Eudistoma ovatum (Herdman, 1886)	Present	Native	250238	n/a

Photo 18 Polycitoridae species

Photo of polycitorid ascidians; a) *Eudistoma elongatum* in New Zealand. Source: M. Page, Earth Sciences New Zealand

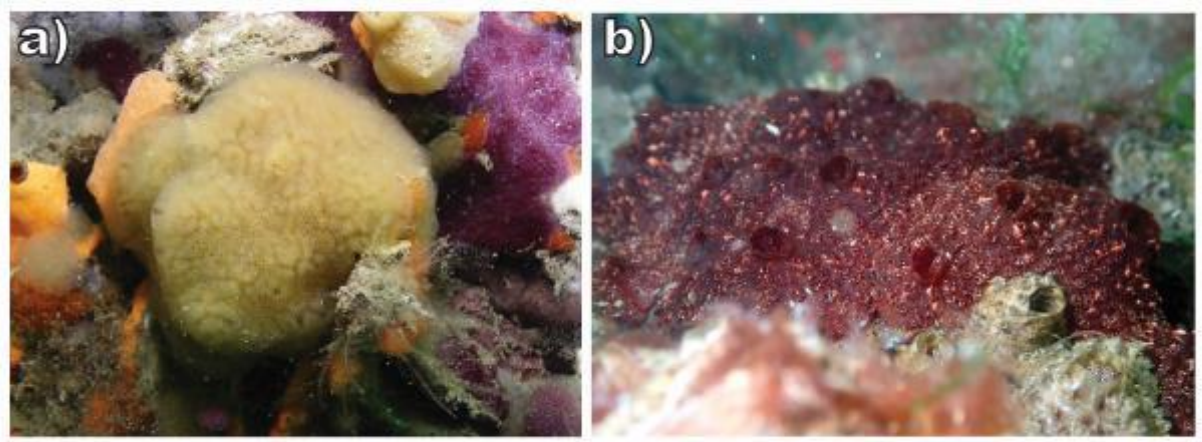
Polyclinidae

All Polyclinidae species are colonial with sexual and asexual reproduction.

Table 13 Polyclinidae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Aplidium australiense Kott, 1963	Present	Native	249820	n/a
Aplidium constellatum (Verrill, 1871)	Not detected	Non-native	251553	n/a
Aplidium phortax (Michaelsen, 1924)	Present	Native	249911	Photo 19 Polyclinidae speciesa
Aplidium solidum (Ritter & Forsyth, 1917)	Present	Native	215889	n/a
Polyclinum constellatum Savigny, 1816	Not-detected	Non-native	215891	Photo 19 Polyclinidae speciesb

Photo 19 Polyclinidae species



Photos of polyclinid ascidians: a) *Aplidium phortax* in the Marlborough Sounds, New Zealand; b) *Polyclinum constellatum* in Australia. Sources a) M. Page, Earth Sciences New Zealand; b) M. Tank

Pyuridae

All Pyuridae species are solitary with sexual reproduction. Many species are harvested and farmed for human consumption (see Section 2.3).

Table 14 Pyuridae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Halocynthia roretzi (Drasche, 1884)	Not detected	Non-native	250680	n/a
Microcosmus exasperatus Heller, 1878	Present	Cryptogenic	103838	n/a
Microcosmus squamiger Michaelsen, 1927	Present	Native	236666	n/a
Pyura cancellata Brewin, 1946	Not detected	Non-native	251254	Photo 1b
Pyura chilensis Molina, 1782	Not detected	Non-native	251256	n/a
Pyura dalbyi Rius & Teske, 2011	Present	Native	564381	n/a
Pyura doppelgangera Rius & Teske, 2013	Present	Native to Tasmania, non-native across rest of Australia	734059	Photo 5b
Pyura pachydermatina (Herdman, 1881)	Not detected	Non-native	251287	Photo 5a, Photo 20 Pyuridae speciesa
Pyura praeputialis (Heller, 1878)	Present	Native	251292	n/a
Pyura stolonifera (Heller, 1878)	Present	Cryptogenic	215927	n/a

Photo 20 Pyuridae species

Photo of pyurid ascidians; a) *Pyura pachydermatina* in Dunedin, New Zealand. Sources: a) F. Vaux, Earth Sciences New Zealand

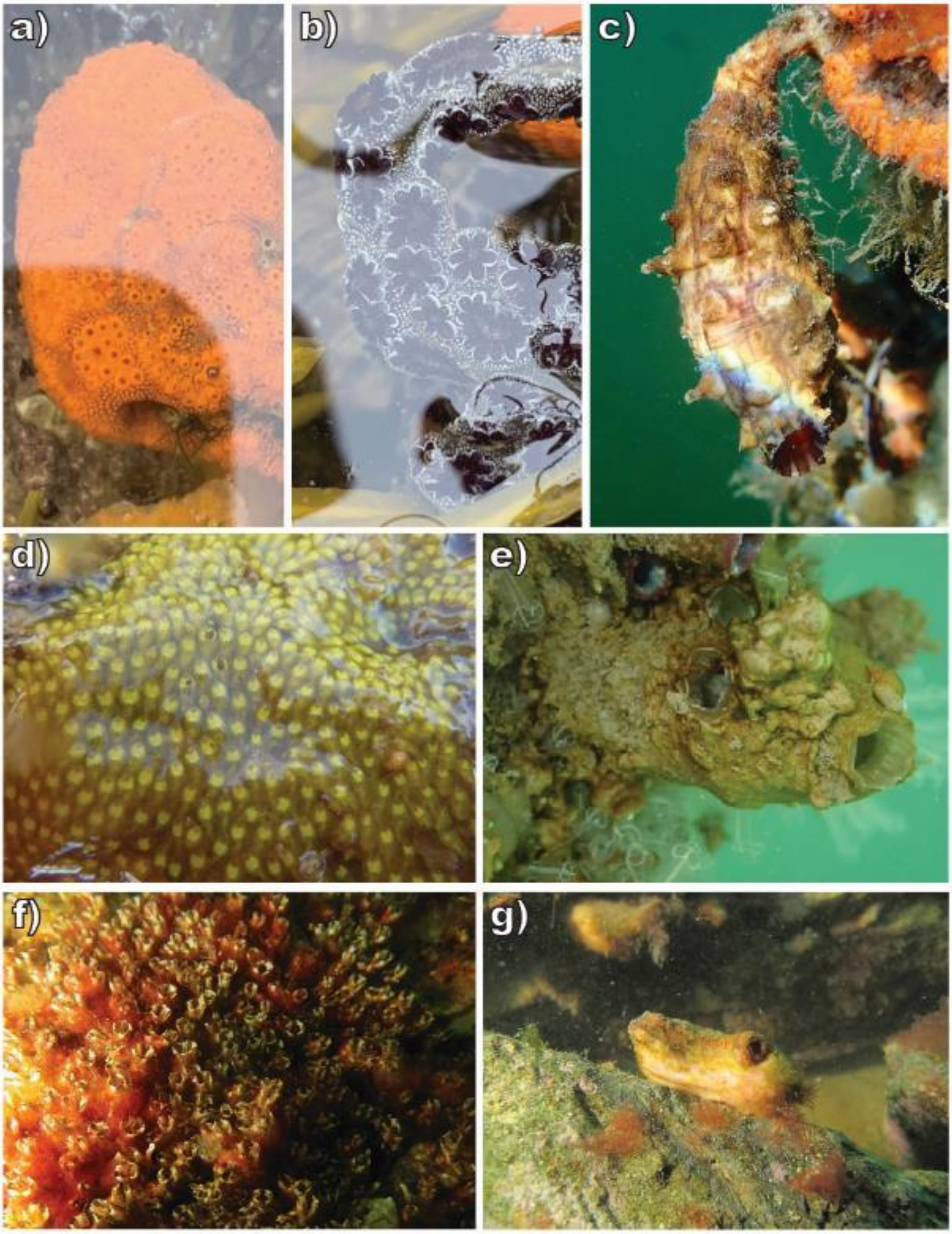
Styelidae

The family Styelidae contains a mixture of solitary and colonial species. *Botrylloides, Botryllus, Polyandrocarpa,* and *Symplegma* species are all colonial with sexual and asexual reproduction, whereas *Styela* species are solitary with sexual reproduction. Some species are harvested and farmed for human consumption (see Section 2.3).

Table 15 Styelidae species

Species and taxonomic authority	Presence in Australia	Invasion status in Australia	WoRMS taxonomic ID	Photo reference
Botrylloides leachii (Savigny, 1816)	Established	Cryptogenic	250081	n/a
Botrylloides giganteus (Pérès, 1949)	Established	Non-native	1502195	n/a
Botrylloides diegensis Ritter & Forsyth, 1917	Not detected	Non-native	252278	Photo 3f, Photo 7c
Botrylloides saccus Kott, 2003	Present	Native	250084	n/a
Botrylloides violaceus Oka, 1927	Not detected	Non-native	148715	n/a
Botryllus schlosseri (Pallas, 1766)	Established	Cryptogenic	103862	Photo 4a, Photo 21 Styelidae speciesb
Cnemidocarpa bicornuta (Sluiter, 1900)	Not detected	Non-native	250333	Photo 1a, Photo 21 Styelidae speciesg
Polyandrocarpa zorritensis (Van Name, 1931)	Not detected	Non-native	103895	Photo 21 Styelidae speciesf
Styela clava Herdman, 1881	Established	Non-native	103929	Photo 2a, Photo 8c, Photo 21 Styelidae speciesc
Styela plicata (Lesueur, 1823)	Established	Non-native	103936	Photo 2a, Photo 21 Styelidae speciese
Symplegma brakenhielmi (Michaelsen, 1904)	Present	Cryptogenic	251435	Photo 4b, Photo 8a, Photo 21 Styelidae speciesd

Photo 21 Styelidae species



Photos of styelid ascidians; a) *Botrylloides violaceus* near Hog Island, Maine, USA; b) *Botryllus schlosseri* near Hog Island, Maine, USA; c) *Styela clava* near Vissschershoek, Netherlands; d) *Symplegma brakenhielmi* from Maria Island, New Zealand; e) *Styela plicata* from Picton, New Zealand; f) *Polyandrocarpa zorritensis* in Nelson Harbour, New Zealand; g) *Cnemidocarpa bicornuta* from Waikawa Marina, New Zealand. Sources*:* a and b) Dr Vaugh Shirley; c) Jean-Paul Boerekamps; d) Javier Couper; e, f) C. Woods, Earth Sciences New Zealand; g) M. Page, Earth Sciences New Zealand

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), the 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. The 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 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 started 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 16).

Table 16 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* *Port Authorities Act 1999*	dpird.wa.gov.au/businesses/biosecurity/aquatic-biosecurity/aquatic-pest-biosecurity-policy
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

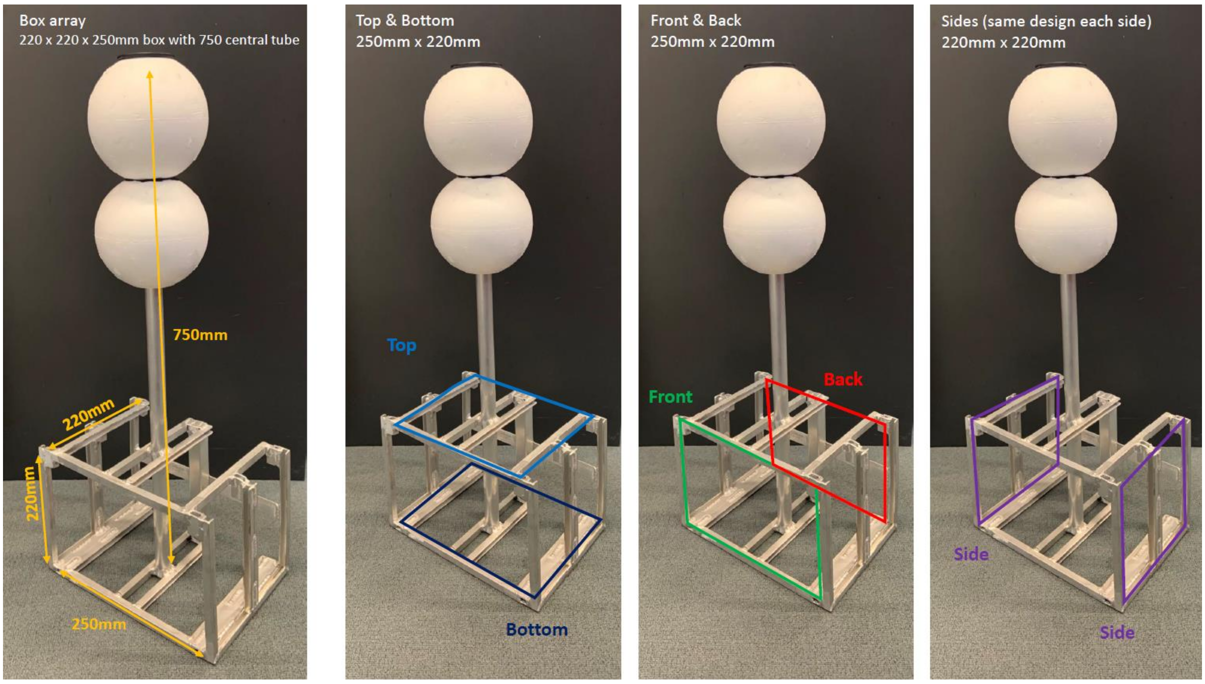
Appendix E: Settlement array designs to sample invasive ascidians

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 ascidians 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 22).

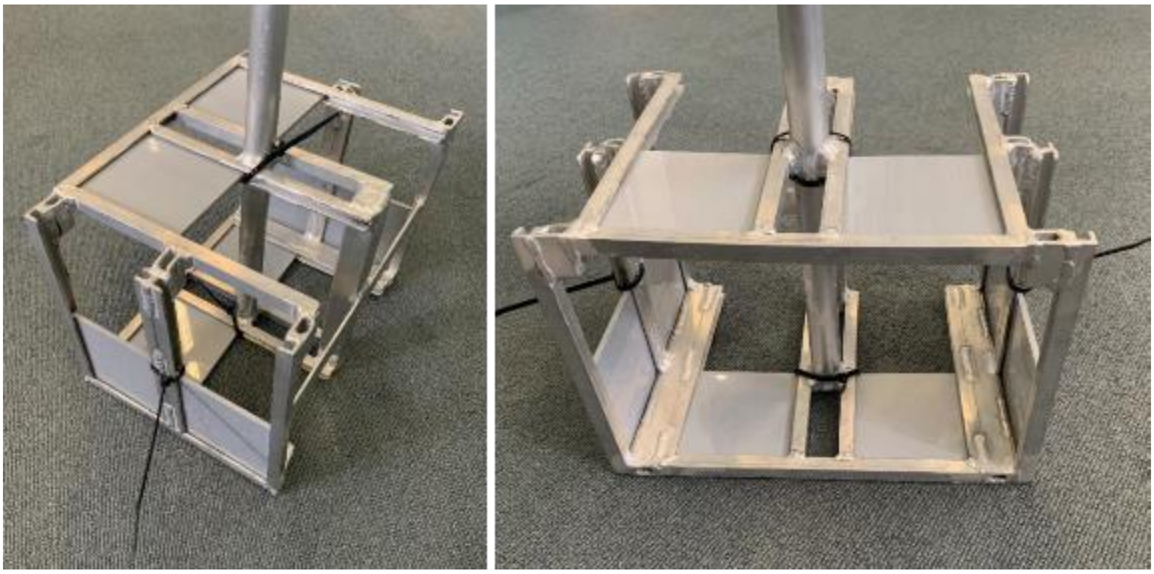
Photo 22 Stepped box array design showing different sides and measurements



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 23). 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.

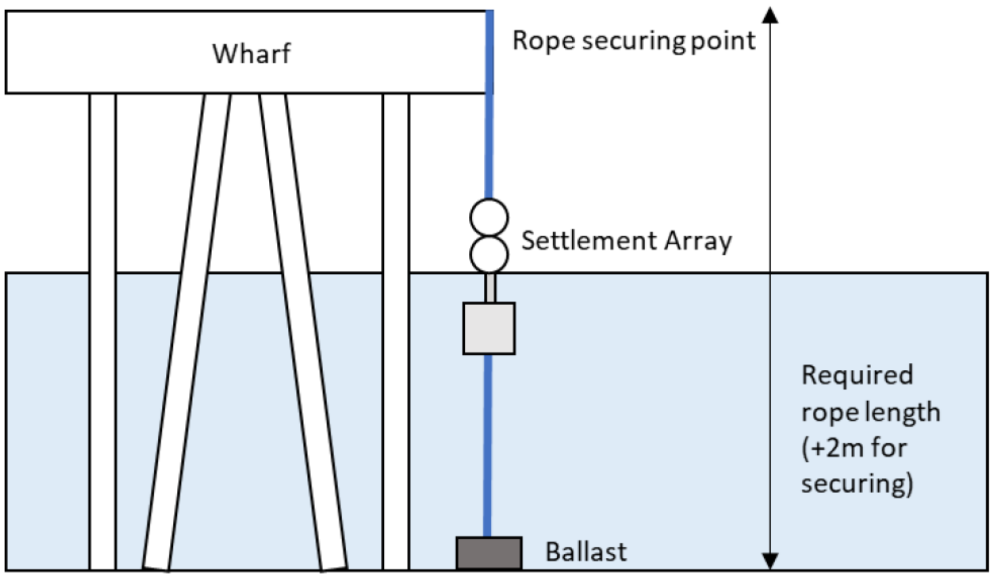
Photo 23 Grey PVC plates (10 cm^2) inserted into U-channels of the box array



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 9). 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 9 Schematic of typical box array deployment



Source: *A*quatic 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 24). 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.

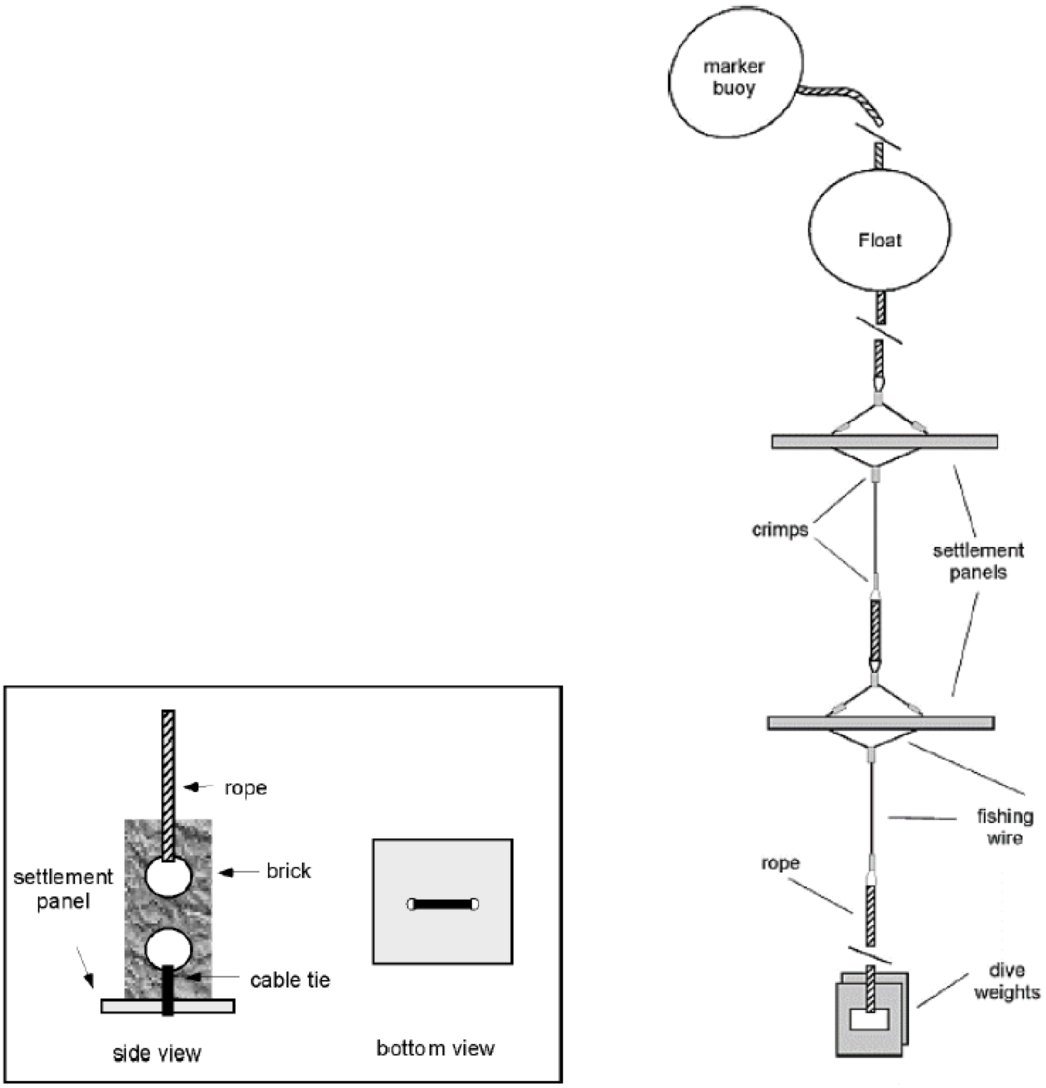
Photo 24 Box array and crab condo ready for deployment (left) and box array deployed in-water (right)

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 10). 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 10 Hanging array design recommended by Sutton and Hewitt (2004)



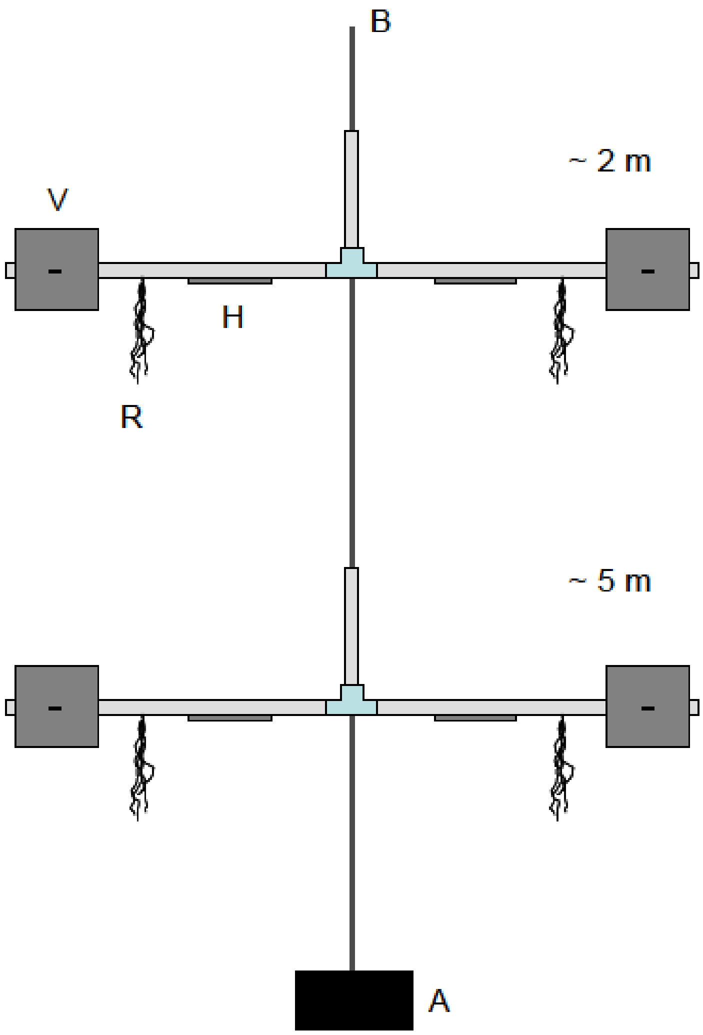
Source: Tait & Inglis (2016)

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 11) 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 11 Double T-unit array design showing vertical plates (V) and horizontal plates (H)

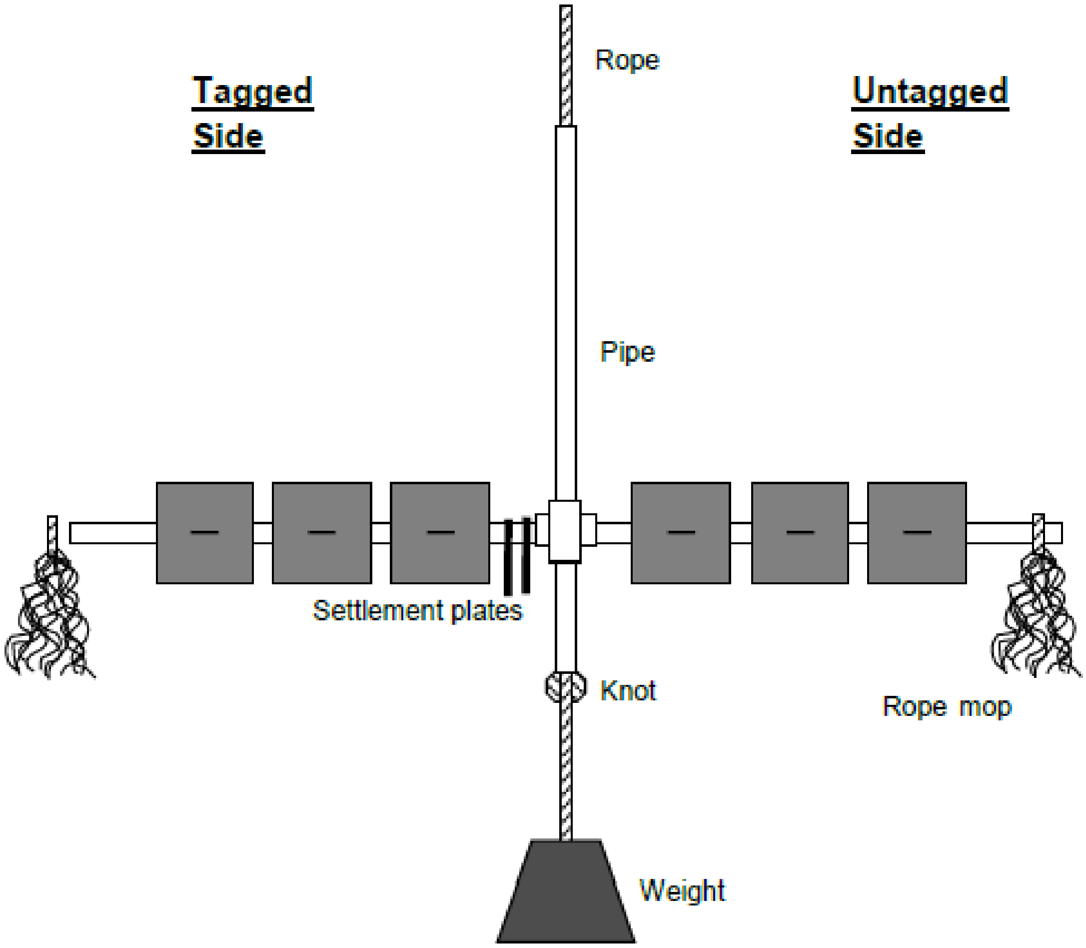


Source*:* Floerl et al. (2012)

Single T-unit array

One variation of the double T-unit design has been used in the Northern Territory (Figure 12), 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 12 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.

Glossary

Term	Definition
Ascidian	Invertebrate marine animals from the Phylum Chordata commonly called ‘sea squirts’. Ascidians have a ‘tunic coat’ and can be solitary or colonial and are a known fouling species.
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
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 measures to mitigate further spread. Native or indigenous plants and animals are not characterised as established marine pest (even if having negative impacts).
Exotic	See ‘marine pest’.

Term	Definition
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.
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 monitoring, 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.
Native species	For the purpose of this manual, native species means any ascidian naturally occurring within a region within Australia.
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.
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 ascidians). 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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