Man-made marinas as sheltered islands for alien marine organisms: Establishment and eradication of an alien invasive marine species

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Abstract The typical tidal range in the west and north-west areas of the Northern Territory, Australia, is 8 m. Four sheltered marinas with double lock gates have been developed to date from the Darwin Harbour estuary, or dug from the shoreline, to provide regulated environments with no tidal range. These sheltered marinas are novel environments and provide habitat islands for colonisation by invasive alien marine species. In March 1999, a fouling mussel, *Mytilopsis* sp., closely related to the freshwater zebra mussel, *Dreissena polymorpha,* was discovered in one of the marinas at densities up to 23,650/m^2. It had reached those densities in less than six months. We describe the colonisation of this and other marinas by the mussel, and the approaches taken to quarantine and eventually eradicate it. Lastly, we discuss the features that may have led to the invasion and present actions that are being taken to reduce the risk of future invasions.

Keywords invasive alien marine species; marine pests; mussels; *Mytilopsis* sp.; marinas; chlorine; copper sulphate; detergent; temperature.

INTRODUCTION

Increased population and expanded tourism in coastal regions has resulted in an increasing number of man-made structures to service the economic, residential, recreational and aesthetic desires of coastal communities. These novel physical habitats or habitat-islands (e.g. piers, breakwaters, seawalls, eutrophic and polluted areas, docks and marinas, boat hulls and ballast tanks) often support assemblages that are distinct from neighbouring communities (Glasby 1999). So long as the novel assemblage is formed from elements of local communities this is not a major concern. However, when novel physical habitats are developed in areas subject to a high influx of alien organisms, the combination could increase opportunities for invasion by alien species and a source for colonisation of adjacent established communities (cf. MacArthur and Wilson 1967).

Boat marinas in particular are novel marine habitat islands in a colonisation corridor. Since 1988 four boat marinas, closed off from adjacent waters by double lock gates, have been built in Darwin, Northern Territory, Australia. On 27 March 1999, CSIRO divers undertaking a survey for exotic species discovered huge numbers of an unidentified mussel in one of these marinas (Bax 1999; Ferguson 2000; Willan *et al*. 2000). The mussel, nominally *Mytilopsis sallei*, is a close relative of the zebra mussel *Dreissena polymorpha*, a species estimated to cost U.S raw water-dependent infrastructure USD18 million in 1995 alone (O'Neill 1996).

Based on literature reports of the environmental and infrastructure damage caused by *D. polymorpha* in the US, and by *Mytilopsis* sp. in Southeast Asia, where it is introduced (Morton 1989), the mussel was seen as a threat to water-dependent marine infrastructure around northern Australia, to a local A$40 million pearl fishery, and to the environment. Given this threat, and the apparent restricted distribution of the mussel, the Northern Territory Government determined that a fast and vigorous response was called for including, if possible, eradication of the mussel. The Northern Territory Government has a history of responding rapidly and effectively to invasions of terrestrial pests that threaten the local (and national) agricultural industries; here, they extended this experience to a marine alien invasive species.

In this paper we describe, the response by the Northern Territory Government and by Australian national agencies to control the new invasion, to reduce the risk of the species spreading in Australia, and to reduce the likelihood of future introductions of this species. We describe the successful eradication effort, discuss the lessons learned from it and further consider the conditions that contribute to invasion of these marine habitat islands.

Following Willan *et al.* (2000), we use *Mytilopsis* as the genus name. However, because there is some confusion in the literature over the species-level identification of *Mytilopsis* species in Southeast Asia (see contrasting views in Morton 1981 and Marelli and Gray 1985), and because the different species may have different environmental limits and potential impacts, we refer to the mussel as *Mytilopsis* sp. in this paper. For legislative purposes, the mussel was referred to as *Mytilopsis* (= *Congeria*) sp. Despite detailed morphological examination of the specimens from Darwin, it is still not clear which species of *Mytilopsis* invaded, which reflects the uncertain taxonomy of the genus.

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Table 1 Sequence of events associated with the eradication of *Mytilopsis* sp. in Darwin, Australia (data from Ferguson 2000).

Date	Event
27 March 1999	Massive infestations of colonising mussel found in Cullen Bay Marina
29 March	Northern Territory agencies and minister informed
30 March	Special meeting of cabinet to pass regulatory amendments and approve expenditure of funds
31 March	Emergency management team convened; three marinas quarantined to prevent further spread of *Mytilopsis* sp.; marina locks dosed with sodium hypochlorite to create sterile plug
1 April	Media and public informed
2 April	Extensive diver surveys began; list of potentially colonised vessels developed
3 April	Copper sulphate tested in Tipperary Waters Estate Marina
4 April	Chlorine treatment of Cullen Bay Marina; Vessel tracking database established (420 vessels identified as “at risk”); treatment of vessels’ internal plumbing tested
5 April	Chlorine treatment of Cullen Bay Marina continued
6 April	Chlorine treatment of Cullen Bay Marina continued; National Taskforce established; 100% kill rate in Tipperary Waters Estate Marina
7 April	Copper sulphate treatment of Cullen Bay; chlorine treatment of Frances Bay Mooring Basin; vessel cleaning protocols released; scientific sub-committee of National Taskforce established
8 April	Copper sulphate added to Frances Bay Mooring Basin; endoscopes used to check internal plumbing of vessels in Cullen Bay Marina
9 April	Further chlorine treatment of Cullen Bay Marina following heavy rain
12 April	No live mussels in Cullen Bay Marina monitoring areas; some cleaned vessels allowed to leave
16 April	Surviving mussels detected on vessels leaving Cullen Bay Marina; marinas closed again and quarantined; intensive diver surveys of marinas; National protocols formally released
17-19 April	Intensive sampling of Cullen Bay Marina detected two live and two recently-dead mussels among hundreds of thousands of dead mussels; copper sulphate added to specific sites in marina
20 April	Cullen Bay Marina reopened at high tide for limited access; resurvey of Tipperary Waters Estate Marina to confirm absence of live mussels
22 April	Resurvey of Frances Bay Mooring Basin to confirm absence of mussels
23 April	Quarantine lifted from all three marinas; marinas re-opened for normal use; monitoring continued
29 April	National Taskforce ceased operation
8 May	21 day “all-clear” issued for all three marinas. Precautionary vessel checking and treatment arrangements remained in place
July	National Taskforce for the Prevention and Management of Marine Pest Incursions established to examine all aspects of alien invasive marine species management
December 23	Taskforce report delivered to government ministers
4 January 2000	Contracts signed for development of comprehensive databases to assist future rapid responses to alien invasive marine species
22 Dec 2000	Web-based toolbox of all documented control measures used against alien invasive marine species completed and online

THE INVASION

Detection

The mussel was discovered in Cullen Bay Marina on 27 March 1999 at densities up to 23 650 individuals/m^2 (Bax 1999; Ferguson 2000; Willan *et al.* 2000)(Table 1). It was not present six months earlier in the dry season baseline survey conducted by the same divers in the same locations (C. Hewitt, CSIRO pers. comm.), indicating that the mussel has the potential for explosive population growth in these marina environments.

Several days later the mussel was also found in low numbers in a second marina, known as Tipperary Waters Estate Marina. This marina had only been recently excavated from dry land and had only six yachts berthed within it. The mussel was found on the hull of one recreational yacht that had come from Cullen Bay Marina, and on the adjacent pilings.

A third marina, Frances Bay Mooring Basin, had also received yachts that had been moored in Cullen Bay Marina. One of these was fouled with the mussel.

Characteristics of the invaded area

Darwin in the Northern Territory of Australia (Fig. 1) is an area of environmental extremes; 8 m spring tides are common, and monsoonal climate provides alternating wet and dry seasons. The extreme tides limit successful settlement of marine species due to the associated strong currents and large expanse of exposed intertidal habitat. Native species are adapted to this environment. Closed mari-

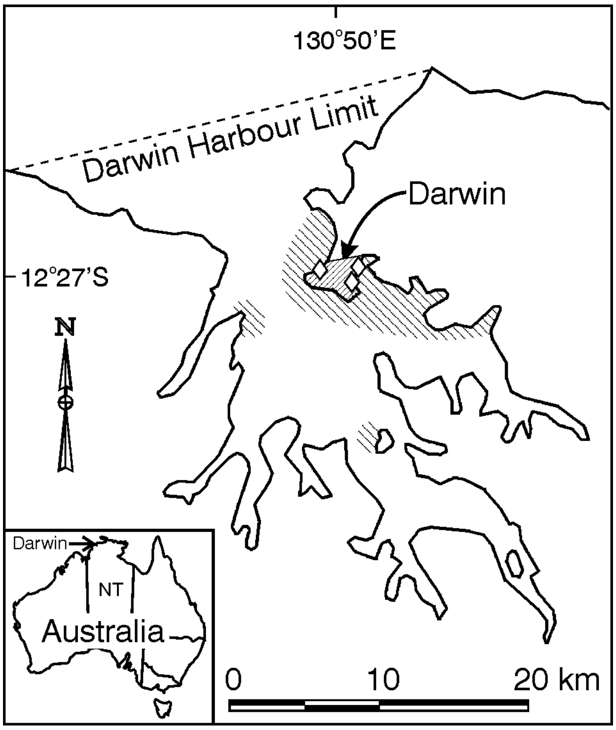

Fig. 1 Map of Darwin, indicating the three marinas (diamonds) that were treated and the areas (hatched) within Darwin Harbour where all man-made structures (wharves, oil rigs, channel markers, etc) and anchorages were surveyed by divers. Gove Harbour (600 km to the east) was also surveyed by divers.

nas prevent the large tidal excursion, and thus provide a novel habitat island that may be more readily colonised by alien invasive marine species.

At the time of the invasion there were three marinas in Darwin (Fig. 1): Frances Bay Mooring Basin, a 250 ML primarily commercial vessel marina with 83 berths that opened in 1988; Cullen Bay Marina, a 600 ML recreational marina with 135 berths that opened in March 1994; and Tipperary Waters Estate Marina, a 150 ML recreational marina with 77 berths that opened in March 1999.

Double lock gates operate to pass vessels into and out of the marinas at all stages of the tide. Depending on season and the level of flushing by the marina operators, the marina can be strongly stratified with an overlying freshwater lens up to a metre deep.

Biology of *Mytilopsis*

The two extant Dreissenidae genera (*Dreissena* and *Mytilopsis*) probably originated in the Ponto-Caspian Basin during the Eocene (Marelli and Gray 1985). The extant five to nine *Mytilopsis* 'species' occur principally in the Americas; one was introduced to north-west Europe in the late 19th century and there is another in western Africa. All species are mytiliform, byssate and epifaunal and inhabitat brackish waters. There are two tropical species, either of which could have invaded Darwin. *M. sallei* occurs naturally in the West Indies, along the Caribbean coast of Central and South America from Yucatan to Venezuela, and in southern peninsular Florida, U.S.A. *M. adamsi* (Morrison 1946) occurs in western Panama. *M. sallei* occurs naturally in a low tidal regime; *M. adamsi* occurs naturally in areas of high tidal range. Both tolerate varied environmental conditions (10-35ºC and 0-80 ppt salinity). Because of the uncertainty about which *Mytilopsis* species was present in Darwin, the wide tolerance of the genus added to concerns over the range of habitats potentially at risk.

Sometime prior to 1929, *Mytilopsis* sp. was reported from Fiji (Hertlein and Hanna 1949). Species identity is still uncertain. It may have been *M. sallei*, entering the Pacific after the Panama Canal was opened (Morton 1981), or *M. adamsi* that occurs naturally in the Pacific and extended its range with the assistance of mail steamships that plied the Pacific between Panama and Australia in the late 19th century using Fiji as a port (Marelli and Gray 1985). *Mytilopsis* has since been recorded from India, Japan, Taiwan and Hong Kong (1967, 1974, 1977 and 1980, respectively)(Morton 1981).

Ripe individuals of *M. sallei* are found all year round in the brackish water of its native range, but it has two periods of intense spawning activity, apparently stimulated by rapid drops in salinity resulting from seasonal freshwater outflow (Puyana 1995). Outside of its native range, nominal *M. sallei* favours disturbed environments, spawns twice a year and may be ambisexual (Karande and Menon 1975), or predominantly semelparous (Morton 1989). Ambiguity in the literature over the reproductive biology of invasive populations could indicate that more than one species has colonised Asian ports. Juveniles from the year's first spawning are mature within a month and contribute to the year's second spawning event (Karande and Menon 1975; Morton 1989). The one month maturity led us to assume that an infected vessel would be able to transmit *Mytilopsis* sp. 30 days after being exposed to a viable population.

PRELIMINARY STEPS

Hazard analysis

Risk assessment provides a framework to weigh the relative costs and benefits of an eradication effort (Bax *et al*. 2001). There are usually four or five stages to a risk assessment (Hayes 1998). The first stage is often to identify all potential hazards associated with a particular event; the second to quantify the risk associated with each hazard. Hazard identification serves an important role itself by providing a checklist of the hazards that need to be considered and (potentially) their relative importance. There is a wide variety of hazard identification techniques. Most of these involve 'workshops' with persons well acquainted with the area or system where the hazards are to be identified. There was insufficient time (or established protocols) to use a workshop approach in this case. Instead, scientists from the CSIRO Centre for Research Into

Table 2 Potential vectors for *Mytilopsis* sp. in Cullen Bay.

Vector	Larvae		Adults	
	Ballast Water	Other Water	External Fouling	Internal Fouling
International and domestic yachts (long & short term residents)	X	X	X	X
Quarantine vessel, dive boats, naval vessels, Fisheries Protection vessel[1]		X	X	X
Ferries	X	X	X	X
Recreational craft (e.g. dinghies, jet-skis, outboards, etc.)		X	X	X
Fishing gear & nets			X	
Buoys/traps/floats[2]			X	X
Loch water		X		
Bay water or substrate samples (e.g. for aquaria, bait)		X		
Flotsam and jetsam		X	X	
Fauna (e.g. birds, crustacea)			X	
Pipe reverse flow (e.g. stormwater overflow, sewage)		X	X	X

[1] These vessels do not hold substantial quantities of ballast water because they don't load and unload large quantities of cargo (some naval vessels might but not the ones in Darwin at the time).

[2] Damaged buoys may hold small quantities of water. Floats are usually porous to some degree hence they may hold water but this is not a viable vector for larvae.

Marine Pests (K. H., R. T., N. B. and Chad Hewitt) conducted an initial hazard analysis for *Mytilopsis* sp. in Cullen Bay Marina.

Egg/sperm and larvae were treated as larvae, while juveniles and adults were treated as adults to reduce the number of hazard analyses required with no perceived loss of hazard identification. A lack of information on larval settlement preferences led us to assume that larvae would act passively in the water column with no settlement preferences, and that juveniles and adults would settle permanently and mature rapidly. These assumptions tend to overestimate potential hazards and are therefore conservative.

Four main ways in which mussels could leave the marinas were identified: in ballast water; other water (e.g. bilge water, anchor well water, etc.); external fouling on the exposed hull, and internal fouling in pipes; and inlets leading off the hull (Table 2). Simple 'fault trees' were constructed for adults carried as external or internal fouling and for larvae carried in ballast or other water (Tables 3 and 4). Hazard management options were then developed (Table 5).

A hazard analysis was also carried out on vessels that had potentially been in an infested area. Four risk categories were identified for areas, and vessels were assigned the risk level of the area they had entered. The hazard analysis suggests that the pelagic larval life-stages of *Mytilopsis* sp. are the most "infectious" and therefore the most likely means of transmission of the organism beyond the infestation. A simple qualitative risk assessment was therefore implemented along the following lines:

Confirmed high risk areas: those areas where spawning had been shown to have occurred; Cullen Bay and Tipperary Waters Estate marinas only.

High risk areas: those areas exposed to an extant population of *Mytilopsis* sp. (i.e. on an infected vessel) and where there had been insufficient in-water surveys or larval/post larval collections (see below) to determine whether spawning had occurred.

Medium risk areas: those areas where a reproductive population of *Mytilopsis* sp. was known to have been (ie on an infected vessel) but, either the source of infection posed a medium risk (i.e. a vessel exposed in a another medium risk area), or extensive and weekly in-water surveys or surveys using larval settlement plates detected no indications of larval settlement.

Low risk areas: those areas that had either been treated or had had two in-water surveys one month apart with no detection of juvenile or adult *Mytilopsis* sp., were subsequently monitored monthly for post larval settlement, and had not received untreated vessels from medium or high risk areas since treatment or completion of surveys.

These categories were used to set priorities for interdicting and treating potentially infested vessels.

Surveys

Twenty-eight divers, supported by surface teams to withstand the strong currents and to keep watch for crocodiles, conducted systematic surveys of all apparently suitable habitats for *Mytilopsis* sp. in the three marinas, around Darwin Harbour and as far afield as Gove Harbour; a harbour with a 1 m tidal range, frequented by visiting international yachts, served by ferry from Cullen Bay Marina, and therefore deemed to be a high risk area (Ferguson 2000). Barges, oil rigs, wharf piles, the naval base and sewage and storm water drains in the three marinas were among the habitats inspected.

Table 3 Hazard analysis for *Mytilopsis* sp. adults in external and internal fouling.

ENDPOINT	NECESSARY EVENTS	
Escape of adult *Mytilopsis* on external and internal fouling vectors	1. Adults remain viable on exit from Cullen Bay.	a. Oxygen remains within tolerable limit b. Sufficient moisture to prevent desiccation
	2a Vector infected with adults in Cullen Bay OR	a. Vector in Cullen Bay during settlement of larvae following period of spawning b. Larvae settle on vector
	2b Vector picks up dislodged adults	a. Adults dislodged b. Adults re-attach to vector
	3. Vector leaves Cullen Bay	a. Vessel movement out of the bay (international and domestic yachts, quarantine vessel, dive boats, ferries, naval vessels, fisheries protection vessel, recreational craft) b. Movement of gear out of bay (outboard motors, buoys/ pots/ floats, fishing gear and nets) c. Other material movement out of the bay (bay substrate, flotsam, other fauna)

Table 4 Hazard analysis for *Mytilopsis* sp. larvae in ballast and other water.

ENDPOINT	NECESSARY EVENTS	
Escape of larvae *Mytilopsis* in ballast water and other water	1. Larvae remain viable on exit from Cullen Bay	a. Salinity remains within tolerable limits b. Temperature remains within tolerable limits c. Oxygen remains within tolerable limits d. Sufficient food sources e. Sufficient moisture to prevent desiccation
	2. Vector infected with larvae	a. Vector in Cullen Bay during period of spawning and prior to settlement
	3. Vector leaves Cullen Bay	a. Ballast water exit from Cullen Bay (international and domestic yachts, ferries) b. Other vessel water exit from Cullen Bay (bilges and other sea water systems of yachts, quarantine vessel, dive boats, ferries, naval vessels, fishing protection vessel and recreational craft) c. Other water exit from bay (leakage from lock, samples of bay water, outboard motors, flotsam)

Table 5 Hazard management options for *Mytilopsis* sp. in Cullen Bay Marina.

POTENTIAL VECTOR	SUGGESTED MANAGEMENT
International and domestic yachts (long & short term residents)	Clean external submerged surfaces Treat internal seawater systems Treat ballast (or residual ballast in empty tanks) Remove domestic yachts from Cullen Bay once cleaned
Quarantine vessel, dive boats, naval vessels, Fisheries protection vessel	Clean external submerged surfaces Treat internal seawater systems
Ferries	Clean external submerged surfaces Treat internal sea-water systems Treat ballast (or residual ballast in empty tanks)
Recreational craft (e.g. dinghies, jet-skis, outboard motors, etc.)	Clean external submerged surfaces Clean and dry internal seawater systems Educate users and repairers on risks
Fishing gear & nets	Clean and dry on removal from bay Educate users on risks
Buoys/pots/floats	Clean and dry Ban removal from bay Educate users on risks
Loch water	Maintain positive pressure into bay Treat or prevent escape of lock water
Bay water or substrate (e.g. for aquaria, bait)	Educate users on risks
Flotsam and jetsam	Dry prior to onshore disposal Prevent escape via lock
Fauna (e.g. birds, crustacea)	Verify the importance of this vector prior to management
Pipe reverse flow (e.g. stormwater overflow, sewage)	Clean Ensure positive pressure into bay

Marina operators record vessels entering and leaving the marinas as they pass through the lock gates. All vessels found to have been in the marinas during the time that *Mytilopsis* sp. was present and capable of spawning (taken to be one month after the August 1998 survey when no mussels were detected) were tracked and located. Survey protocols to inspect vessels were developed in conjunction with boatyard operators and the fishing industry. Particular attention was given to the hull surface, ropes, chains, anchors, seawater inlets and internal water systems. Approximately 250 vessels were inspected by divers (Ferguson 2000). Divers used 3 m and 22 m long endoscopes to survey the interior spaces (e.g. water intakes and outlets).

Laboratory trials of treatment options

Chlorine and chlorine dioxide are frequently used to remove *D. polymorpha* from water-based infrastructure in the US (Boelman *et al*. 1997), and it seemed likely that they would be suitable to remove *Mytilopsis* sp. from the marinas. The relatively easy availability of chlorine (as hypochlorite), which is used to clean swimming pools, made this an attractive option. Chlorine dioxide was also available, and in theory should have been more effective than hypochlorite, so this was a second option. However, *D. polymorpha* is a freshwater species, and there was no guarantee that chlorine would be equally effective against the marine *Mytilopsis* sp. Therefore four additional treatments were tested: copper sulphate; a patented organic copper complex; hot water; and detergent. Non-oxidising chemicals (e.g. quaternary ammonium compounds), reported to kill the zebra mussel in the U.S, were not tested as sufficient quantities for treatment could not be located in Australia.

All tests were conducted in triplicate on Cullen Bay Marina mussels held in 2 *l* glass beakers of Cullen Bay Marina water at the Northern Territory University. The salinity was 18 parts per thousand (ppt), pH 8.1, turbidity 2-3 nephelometric turbidity units; dissolved oxygen 90-100%; temperature 29-33°C (ambient). Salinity was lower than seawater as it was at the end of the wet season and the locked marinas had significant freshwater inflow. Beakers were covered with a watch glass cover to reduce evaporation. Mussels were obtained fresh from the marinas and were not fed. Approximately 30 individuals with maximum shell length of 1 to 1.5 cm were used in each beaker. The LT_{100} (time to achieve 100% mortality) was determined for each treatment. Death was determined as gaping shells unresponsive to touch. The LT_{100} was used in preference to the LT_{50}, more commonly used in toxicity trials, as we required a treatment that would kill all of the mussels. Chemical concentrations were checked twice a day (colorimetric method for chlorine; ICP Mass spectrometry for copper), and additional chemicals added if the concentration had dropped below the test level. Laboratory conditions were not ideal standardised conditions because they were hastily set up to identify an effective treatment within days, however they were thought to more closely represent the conditions that would occur when treating a marina.

Calcium hypochlorite

At least twice-daily measurement of chlorine concentration showed that the nominal concentration in the beakers were difficult to maintain – in fact 12 hours after adjustment of concentrations to 12 or 24 mg/*l*, concentrations weer approaching <1 in the day and 5 mg/*l* at night. This was expected as chlorine is unstable in water and exposure to light and elevated temperatures accelerates the reduction in chlorine concentration. Chlorine concentrations were adjusted after each measurement to maintain the test concentration. The time to 100% death (LT_{100}) ranged from >290 hours at a nominal concentration of 0.0, 0.6, 1.2 and 6.0 (mg/*l*) to 111 hours at a nominal concentration of 12.0 (mg/*l*) and 90 hours at a nominal concentration of 24.0 (mg/*l*). From our experiments, we thought it likely to prove difficult to maintain these concentrations in the infested marinas.

Chlorine dioxide

From literature reports for zebra mussels, we expected chlorine dioxide to be more effective at killing the mussels than hypochlorite. However the protocols provided by the manufacturer for activating the stabilised chlorine dioxide solution did not work – hydrochloric acid activation resulted in the complete loss of chlorine dioxide in <15 hours and citric acid activation did not activate the chlorine dioxide at all. All further tests with chlorine dioxide were abandoned as we did not have the time to work out the correct activation procedure.

Copper sulphate

Copper sulphate was tested on the basis of its common use to kill invertebrates in aquaculture operations. Copper was added as copper sulphate to give a nominal (and subsequently measured) concentration of 1 mg of copper/*l*. In seawater (35ppt) the concentration of Cu is also controlled by copper hydroxide solubility and the saturated concentration in seawater is 2 µM (0.126 mg/*l*). However due to the low salinity water in the marina a concentration of 1 mg/*l* could be maintained. The LT_{100} for copper sulphate was 38 hours.

Copper organic complex

Organic complexes of copper are generally considered to be non-toxic or have lower toxicity than free copper ions. This was confirmed in our trials, which were ended after 48 hours when mussels were still alive in the two treatments (0.5 and 1.0 mg/l).

Combined calcium hypochlorite/copper sulphate

This experiment, designed to simulate possible field trials, used a nominal chlorine concentration of 12mg/*l* chlorine (see notes under calcium hypochlorite experiments) for either 24 or 48 hours followed by addition of 1 mg/*l* Cu. Both treatments resulted in a LT_{100} of 96 hours.

Detergents

Detergent was tested following observations of its lethal effect on marine life in aquaria, and with a view to using it to clear the internal plumbing of vessels. Domestic detergent (1% v/v) in seawater of 13 and 33 ppt salinity gave LT_{100}s of 24 hours. Industrial detergent (Conquest, 1% v/v) in 19 and 33 ppt salinity seawater gave LT_{100}s of 7 hours.

Temperature

Mussels were tested in beakers placed in temperature controlled water baths and held at 40, 50 and 60°C. The LT_{100}s were >120, 30 and 30 minutes, respectively.

THE RESPONSE

Legislative powers and coordination

The Northern Territory Government took a 'whole of government' approach involving all departments, coordinated by the Assistant Chief for Police, Fire and Emergency Services and overseen by the relevant minister. The Minister and the Chief Executive Officer of the Department of Primary Industry and Fisheries were briefed on 29 March 1999, two days after the mussel was first observed (Table 1). The Northern Territory cabinet was informed at the same time. The following day, a special meeting of cabinet was held to amend the Northern Territory *Fisheries Act 1988* to list *Mytilopsis (=Congeria)* sp. as an aquatic pest; to give aquatic pests the same status as diseases and contaminated fish; to decree that restricted areas apply to aquatic pests; and to declare the marinas to be restricted areas and prohibit the movement of aquatic life from these areas (Ferguson 2000). The amendments to the Fisheries Act 1988 were gazetted the following day (31 March 1999) and the three marinas quarantined using a combination of this act and the *Quarantine Act 1908*.

A Northern Territory Taskforce was set up with units responsible for media, vessel tracking, emergency services, health, diving/survey, eradication, biology and treatment. Seven Northern Territory and two national organisations were involved in the local eradication (see acknowledgements for full listing); a further seven national organisations and the States were involved in the national response.

Twice daily meetings of the Taskforce kept all members informed and enabled the rapid identification of priorities and the necessary resources to meet them.

Media issues and community response

A media team was established at the start of the response, using experts from the Northern Territory Police, Fire and Emergency Services accustomed to dealing with disaster response. Immediately the marinas were quarantined, there was pressure from concerned residents, vessel owners, tourism operators and marina businesses to limit the response, especially as the quarantine went into effect just before the Easter holidays. Conversely, local aquaculture businesses and conservation groups wanted assurance that the quarantine and treatments be sufficient to ensure that the eradication was successful. The media group kept the local community, the nation, and international interests informed with daily press releases; regular community fliers; community meetings at Cullen Bay Marina involving the Northern Territory Primary Industry and Fisheries Minister and senior Taskforce members; a public telephone hotline; and a web site.

The work of the media team was critical in gaining community and stakeholder support. The team ensured that consistent and informative messages were provided on Taskforce activities. Press releases and media events were managed so that a new topic was presented each day by authoritative figures and informed scientists, reducing the need for the media to look elsewhere for the day's story. The involvement of national (CSIRO's Centre for Research on Introduced Marine Pests) as well as local scientists in the eradication programme and media interviews, facilitated community and stakeholder engagement in the issue by emphasising that all available resources were being accessed.

Quarantine

Colonised marinas were quarantined on 1 April 1999; five days after the mussels were first observed in Cullen Bay Marina and three days after their identification was confirmed. No vessels inside colonised marinas were permitted to leave until the marinas were declared free from the mussel. Once the three marinas had been quarantined, locally available sodium hypochlorite was added to the short (<40 m) channel between the two lock gates separating each marina from the ocean. This quarantine was used to prevent larval *Mytilopsis* sp. from leaving the marinas alive and to kill any *Mytilopsis* sp. occurring in the channel.

Chemical eradication

Marinas

Treatments for the marinas proper needed careful consideration, due to both their size and usage. The largest, Cullen Bay Marina, had the highest densities of the mussel, up to 23,650/m^2 (Willan *et al.* 2000), compared to 6/m^2 (Ferguson 2000) in Tipperary Waters Estate Marina. None were sighted in Frances Bay Mooring Basin (although an infested yacht that had recently been in Cullen Bay Marina was found in the Basin). Treatment was therefore focused on the Cullen Bay Marina and the vessels still moored there. The smaller Tipperary Waters Estate Marina was used as a field experimental site.

Based on US experience with the freshwater *D. polymorpha* (Boelman *et al.* 1997), chlorine was the preferred option for chemical eradication. It was estimated that several hundred tonnes of chlorine, in the form of liquid sodium hypochlorite, would be needed to raise the larger 600 ML Cullen Bay Marina to 10 ppm free chlorine, and hundreds of tonnes of sodium hypochlorite were shipped to Darwin from chemical plants around Australia. Estimates were of necessity imprecise as we could not accurately estimate the likely loss of chlorine through evaporation and being bound to organic matter. Large pumps were used to aerate the 12 hectares of water in Cullen Bay to raise the oxygen levels, break up the stratification of the salt and freshwater layers, and disperse the chlorine. The first load of sodium hypochlorite was added to Cullen Bay Marina on 4 April 1999, three days after it was quarantined. Concentrations were tested daily thereafter and additional sodium hypochlorite added as necessary to keep concentrations at about 10 mg/*l*.

Following early laboratory data showing the efficacy of copper sulphate, 0.5 tonne was added to 150 ML Tipperary Waters Estate Marina on 3 April 1999 – producing a maximum final 0.45 µm filtered, concentration of 0.8 mg/*l* and a total copper concentration of 1.5 mg/*l* (Parry *et al.* 1999). Measurements at top and bottom of the water column at five sites throughout the marina showed the copper was well mixed with uniform concentrations throughout. The concentration dropped rapidly after significant freshwater inflow to the marina on 8 April with the filtered and total Cu concentrations stabilising between 0.2 and 0.3 mg/l for the following two months. No sodium hypochlorite was added to this marina. A complete census of Tipperary Waters Estate Marina conducted by divers on 6 April 1999, found only dead *Mytilopsis* sp. – this was three days after the addition of copper sulphate. All mussels attached underneath foam panels floating on the surface were dead.

Meanwhile, daily, non-quantitative observations of the mussels in Cullen Bay Marina, and quantitative monitoring of caged mussels, showed that many mussels were surviving the chlorine treatment. Additionally, death rates in the laboratory were not as high as had been hoped for (see section on laboratory results). Laboratory tests and the trial of copper sulphate in Tipperary Waters Estate Marina indicated that copper sulphate was a more effective method to kill *Mytilopsis* sp. than chlorine in these marinas. Copper sulphate was subsequently used in conjunction with chlorine at Cullen Bay and Frances Bay marinas; chlorine's role was seen as primarily to reduce the organic load so that more free copper would be available in the water column. Powdered copper sulphate was added to the water at the aeration pumps to aid its dissolution.

In total, 187 tonnes of liquid sodium hypochlorite and 7.5 tonnes of copper sulphate were added to the three marinas over two weeks (cited in Willan 2000). Sodium hypochlorite was added to Cullen Bay Marina and Frances Bay Mooring Basin prior to addition of copper sulphate. There was no similar pre-treatment of the Tipperary Waters Estate Marina. A maximum final, dissolved (<0.45 µm) concentration of copper of 0.8 mg/*l* was reached in Tipperary Waters Estate and Frances Bay Mooring Basin, while the concentration in Cullen Bay reached 0.5 mg/*l* (Parry *et al.* 1999). The copper concentrations in all three marinas remained uniform throughout the water column, with no stratification observed due to the mixing with aeration pumps and the running of vessels' engines in the marinas during the treatment. The maximum concentrations were only maintained for approximately two days before the levels began to decline due to various precipitation, adsorption and complexation processes in the water column.

Local vessels

Vessels inside the marina were treated at the same time as the marina – external hulls were treated by the surrounding water. Interior plumbing on all vessels was treated by running the relevant pumps or engines and adding copper sulphate solution or detergent to pipes with standing water. Where the owner was unavailable, Fisheries Officers entered the vessels and performed the necessary tasks.

Colonised vessels outside the marinas were either hauled out and cleaned at the nearest facility that had been approved to clean vessels and dispose of the mussels without risking further colonisation, or taken into one of the three colonised marinas for treatment. Two moorages outside the marinas, but still in the Darwin area, had received a total of six colonised vessels. Diver surveys detected no mussel populations at these moorages.

National taskforce

A national taskforce was set up and coordinated by the Commonwealth Department of Agriculture, Forestry and Fisheries – Australia. A scientific sub-committee was set up to develop national protocols for treatment of vessels and for anchorages at potential risk of secondary infestation, which included all those across northern Australia between Fremantle, Western Australia and Sydney, NSW. The sub-committee took an epidemiological approach in developing the protocols. *Mytilopsis* sp. was treated as an infectious disease with an incubation period of 30 days – this period being the minimum reported time for this mussel to become reproductive post-settlement (Karande and Menon 1975; Morton 1989). Any area or vessel which came into contact with an infested vessel after the 30-day incubation period was assigned the same level of infestation risk as the original vessel. An exposed vessel or area was considered infested until proven otherwise.

All told, 223 vessels were within the three marinas and another 197 had left the contaminated areas and put to sea during the time that the marinas were exposed to the mussel (Ferguson 2000). It was therefore urgent that exposed vessels and the areas that they had visited be identified, surveyed and, if necessary, treated to prevent the further spread of this mussel. A database was established by Northern Territory Police and the Australian Quarantine and Import Service to track vessels that had been exposed to *Mytilopsis* sp. but had left the marinas. This database grew to include information on the infection status and whereabouts of 743 potentially-exposed and exposed vessels.

Invoking this emergency action exposed numerous problems in tracking small vessels, and especially recreational yachts, which has since been addressed by a national marine pest task force (SCC/SCFA 1999). When located, the vessels were either examined in the water by divers or removed from the water for examination under a mixture of State and Commonwealth legislation, that was sometimes found insufficient to allow the preferred treatment options. Where there were no safe local facilities to inspect a potentially-contaminated vessel (e.g. the Cocos Keeling Islands off Western Australia), the contaminated vessel was kept away from shore and freshwater influence.

Fifty seven fishing vessels had left the Frances Bay Mooring Basin shortly before it was quarantined. These 57 vessels were part of the 137-vessel Northern Prawn Trawl fleet that would disperse throughout northern Australia at the end of the fishing season. The vessels come into contact around motherships, providing a serious risk of secondary exposure to *Mytilopsis* sp.. Recalling all exposed vessels to port for treatment (in the 30 days incubation period) was not acceptable to industry during the limited prawn season. Instead the Australian Fisheries Management Authority contacted all vessels at sea requiring that they stay at sea until all the 57 exposed vessels had been determined to be clean. Divers surveyed the exposed vessels at sea and declared them clean. Individual cases of exposed vessels returning to shore for mechanical or medical emergencies were dealt with on a case by case basis that minimised further exposure of the coastline to the mussel.

Monitoring

Mytilopsis sp. was only found in two marinas – Cullen Bay Marina and Tipperary Waters Estate Marina – and on vessels originating from Cullen Bay Marina. Three separate diver surveys of Frances Bay Mooring Basin failed to find *Mytilopsis* sp. on either wharf pilings or on vessel hulls. This marina was treated as a precautionary measure because a vessel with mature *Mytilopsis* sp. had been found there and was cleaned on the hard standing area.

Divers, trained to dive in industrial situations, monitored 20 locations within Cullen Bay Marina to assess the efficacy of the chemical treatments as part of the marina clearance and re-opening process. All available habitat was searched in these 20 areas, including storm drains, the inside of debris, etc.

In addition, mussels were suspended in cages at up to three depths (1, 2 and 3 m as available) at 10 locations in Cullen Bay Marina to monitor quantitatively the efficacy of the chemical treatments. Each cage contained approximately 100 mussels. The cages were removed twice daily and the

Table 6 Total number of live, and percentage dead, *Mytilopsis* sp. held in 19 baskets at 10 locations and three depths (1, 2 and 3 m below surface as available) in Cullen Bay Marina. The first addition of chlorine to the marina was on 4 April (Day 0). Copper sulphate was first added on 7 April (Day 3).

	Day								
	-2	-1	0	1	2	3	4	5	6
Number	2149	2149	2149	2146	1767	1297	863	351	0
Average % dead	0	0	0	0	17	38	59	84	100
Min % dead	0	0	0	0	0	4	9	68	100
Max % dead	0	0	0	1	44	68	85	95	100

condition of the mussels assayed for responsiveness to physical probing.

The first deaths in the mussel cages occurred one day after the addition of hypochlorite, but mortality rates varied widely between cages (Table 6). On average, 38% of caged mussels were dead three days after the first addition of hypochlorite, at which time copper sulphate was added. Six days after the first addition of hypochlorite, and three days after the addition of copper sulphate, all mussels in all cages were dead.

Diver surveys in Cullen Bay Marina found no live mussels on 12 April, eight days after the first addition of chlorine and five days after the addition of copper sulphate. However, on 16 April, four days after no live mussels were found at the monitoring sites, live mussels were observed on a vessel slipped for maintenance after leaving Cullen Bay Marina. Subsequent diver surveys (17-19 April) found two live and two recently dead (shell open but flesh not decomposed) mussels among the several hundred thousand dead mussels collected from the 20 locations around the marina and inspected by hand. The areas where the two live mussels were found were treated with additional copper sulphate. No further live mussels were subsequently found in any marina or on any vessel.

CONFIRMING THE ERADICATION

The area immediately outside the infected marinas, and moorages and ports to which high risk vessels had been tracked (sometimes in other states), were monitored for settling mussels using larval settlement plates for up to 12 months after the chemical treatment of Darwin's marinas. Larval settlement plates were used because the planktonic larval distribution of the mussel would distribute the larval mussels broadly, increasing the chances any reproductive population would be detected.

No juvenile *Mytilopsis* sp. were found on larval settlement plates inside or outside of the marinas in Darwin or at the major ports catering for recreational yachts in Queensland or northern Western Australia. After 12 months the eradication of this invasion of *Mytilopsis* sp. was considered complete.

DISCUSSION

The rapid response to, and subsequent eradication of, *Mytilopsis* sp. by the Northern Territory Government assisted by national agencies was not only a salutary lesson for Australia on the dangers of invasive marine alien species entering tropical Australia, but also the first demonstration that successful action against invasive marine alien species was possible. The eradication operation directly involved over 280 personnel and cost in excess of A$2.2 million, excluding personnel costs. The cost was considered cheap in the light of potential damage by *Mytilopsis* sp. to tropical Australian marine industries and the environment. The chemically-treated marinas were artificial environments already polluted from maritime activities and the temporary loss of their fauna was seen as inconsequential in comparison to the threat to the northern Australian coast.

There are several lessons to be learned from this exercise.

Planning

There may be little time to respond to the invasion of a marine alien species; options available to eradicate or control a marine invader rapidly diminish over time if the invader spreads to additional areas. In a maximum of six months (since the first survey of the marinas, at which time *Mytilopsis* sp. was not present in detectable numbers) *Mytilopsis* sp. went from a presumed single population on a visiting vessel to colonising two marinas and reaching densities of up to 23,650 individuals/m^2. If the invasion had not been detected quickly, it is likely that *Mytilopsis* sp. would have established viable populations outside the closed marina environment; our observations at other tropical yacht anchorages indicate many appear to be well suited to the species. Underwater gas arc welders, temporary covering with gravel or sand, and temporary covering with plastic (containing biocides) were proposed as methods to heat, smother, or poison the *Mytilopsis* sp., respectively, if they were discovered outside a closed marina. However, none of these techniques would have been suitable for a dispersed population in an open environment. This problem was understood very early in the programme, which prompted action to ensure that any spread of *Mytilopsis* sp. was detected early, that invaded areas were quarantined immediately, and that eradication proceeded soon thereafter.

Local and national coordination was essential for a comprehensive response, but in practice had to be established quickly and in an *ad hoc* fashion. The system worked because everyone understood the scale of the problem, and an effort was made to ensure that all relevant groups had the option of participating. There were difficulties finding appropriate State (Territory) or Commonwealth legislation to support the quarantine and treatment of privately owned marinas and vessels. The Northern Territory Government had to amend their legislation to give them the power to respond. Amendments occurred within three days of the outbreak being detected – even so there was confusion over the legislative powers available, who could exercise them and who they could be delegated to. The issues of liability coverage for officers and compensation to owners were never completely resolved.

Subsequent to *Mytilopsis*, two national ministerial councils jointly established a National Taskforce on the Prevention of Marine Pest Invasions (SCC/SCFA 1999) to address the structural inadequacies of the *ad hoc* system. Immediate recommendations that have since been enacted include: establishment of a National Introduced Marine Pests Coordination Group to oversee development of interim arrangements and to develop long-term response options; establishment of a Consultative Committee on Introduced Marine Pest Emergencies to provide coordination of rapid responses in the event of a new alien invasive marine species emergency; development of information systems to speed response to new invasions; clarification and updating of legal powers for responding to alien invasive marine species; and establishment of cost-sharing arrangements between the States and the Commonwealth to fund emergency response to future alien invasive marine species. More complex issues, such as the development of a system to track small vessels, are still being considered.

Risk assessment, when implemented properly, is rigorous and systematic but also time-consuming. In this instance of rapid response, there was insufficient time for a complete risk assessment, but the hazard assessment proved useful in identifying major threats to a successful response. An approach, termed 'Infection Modes and Effects Analysis (IMEA)' has been specifically developed to identify and rank the hazards associated with small craft as potential vectors of alien invasive marine species (Hayes in press). A similar process is needed to identify and rank the hazards associated with the outbreak of an alien invasive marine species in a new area. In this instance the hazard assessment identified particular habitats (e.g. storm water drains and internal plumbing on vessels) that were subsequently targeted by the Northern Territory Government during the chemical eradication.

Rapid access to information is a necessity in responding rapidly. This is true both for the scientists and managers involved, and for the community, to ensure their support for the action. With regard to technical details, we were able to access and distribute information relevant to *Mytilopsis* sp. by querying the Sea Grants National Aquatic Nuisance Species Clearinghouse for information on eradication options for the zebra mussel. The rapid response to our query was extraordinarily valuable in rapidly assessing practical options. Subsequently, Australia has recognised the need for easy access to information on a variety of potential invasive marine species, and is about to launch an online National Introduced Marine Pests Information System to provide similar information for alien invasive marine species currently in, or likely to arrive in, Australia. A web-based toolbox of control options has already been developed. This toolbox provides a readily available source for all documented control options for the different taxa, contacts, suppliers and legal restrictions on their use (Bax and McEnnulty 2001).

The success of the eradication programme was due in part to strong community support for the effort. This was facilitated by having a full-time public relations team assigned to the problem, which ensured that media and relevant stakeholders were provided updated information daily and by ensuring that public statements regarding the infestation and eradication effort were handled by only a few designated spokespeople. Public acceptance of the effort was also facilitated by having the response action well embedded in science. Although the rapid and in some respects non-rigorous nature of the experiments to develop effective chemical treatments was clearly explained to the public, the fact that this effort was guided by a substantial literature on zebra mussel control efforts and was undertaken by a team consisting of both local (NT University and government) and national (CSIRO's Centre for Research on Introduced Marine Pests) experts lent the effort essential credibility.

This public credibility also depended, in part, on good luck – the detection of the mussels in closed marinas where chemical treatments were an option. If we are to extend the success of this eradication to include future invasions of alien marine species in more open environments, we will need to expand available treatment options to include: more specific biocides (e.g. ones that would only affect molluscs); engineering developments that would restrict the action of chosen control options to the target area; and failing that, highly-specific biological control agents that can act over a wide area.

Secondary Exposure

The potential for the Darwin eradication attempt to spiral out of control was the greatest through secondary exposure – one contaminated vessel entering an uncontaminated marina (or fishery) and contaminating tens of other vessels that subsequently dispersed to contaminate new vessels and areas. Secondary contamination of a vessel (in this case a submarine) exposed to spawning mussels (*Mytilus galloprovincialis*) on an unsuccessfully cleaned vessel (the battleship *USS Missouri*) has been observed (Carlton 2001). Secondary contamination was a real risk in the Darwin exercise.

The 57 potentially-exposed fishing vessels that left Frances Bay Mooring Basin days before the quarantine was announced, joined the 137-vessel Northern Prawn Fleet, serviced by motherships, round which the vessels congregate to unload their catch. The 137 vessels disperse to ports throughout northern Australia once the season is over. If these vessels had carried *Mytilopsis* sp. to their home ports, the goal to eradicate this mussel from Australia would have been made much harder – probably impossibly so. Because the fishing vessels carry satellite-linked vessel monitoring systems and can be contacted by the Australian Fisheries Management Authority, the 57 vessels could be contacted and surveyed. Fortunately, no contaminated vessels were found and the risk was never realised. Tracking domestic and international recreational vessels through ports throughout mainland Australia and Australia's offshore islands was not as easy. Again we were fortunate that the vessels that were tracked and surveyed were found to be uncontaminated.

Effective quarantine is a prerequisite for an eradication exercise. If *Mytilopis* sp. had colonised a marina without lock gates, where vessel names were not recorded as they passed out of the marina, then tracking and locating exposed vessels would have been impossible.

Prevention

The tidal regime in Darwin is extreme, with 8 m spring tides. Extreme tidal variation does not easily allow stable marina platforms for mooring boats and so all marinas are closed off to some extent from the sea by double lock gates, that are opened for boat passage as necessary, and to flush entrained water on an irregular basis. Depending on the degree of flushing, there can be strong stratification in the marinas during summer monsoonal rains with a 1 m thick lens of freshwater overlying marine water. Many marine organisms that have colonised the upper few metres of underwater structures during the dry season will die when exposed to continuous freshwater during the wet season, unless there is adequate flushing of the marina. New habitat will be exposed to recolonisation by local organisms and new colonisation by exotic organisms brought in on (or in) boats passing through on their way from exotic ports.

Marinas, especially closed marinas such as those in Darwin, can act as marine habitat islands. They provide novel habitat and are situated in the middle of increasingly active transport corridors for marine organisms. In 1967, MacArthur and Wilson introduced the still influential equilibrium theory of island biogeography. This addresses the question of whether the number of new invasions to an area is primarily a function of the size of the island (habitat) or the extent of immigration. To rephrase this in the context of marine habitat islands such as marinas, the question becomes whether the number of new invasions is primarily a function of the size or types of habitat or a function of the frequency of inoculation. Programmes aimed at reducing the risk of marine invasions have in general not taken account of this ongoing debate. Instead it is assumed that reducing the number of new invasions to an area is of paramount importance, and major efforts both nationally and internationally have been directed at reducing the entry of alien invasive marine species – especially in ballast water. *Mytilopsis* sp. has been detected on three foreign fishing vessels and two visiting international recreational vessels since the eradication was completed. It seems inconceivable that the Cullen Bay invasion was the first instance of *Mytilopsis* sp. arriving in Darwin, yet the most likely scenario for this invasion is that it developed from a single inoculation in a closed marina. It subsequently spread to a second closed marina, but repeated diver surveys failed to find any populations outside of these marinas. This suggests that reducing the risk of marine invasions may require management of the receiving environment in addition to reducing the frequency of inoculation.

Reducing the risk of future invasions

Australia was lucky that this first successful establishment of *Mytilopsis* sp. in Australian waters was in closed marinas. If it had occurred in the open waters of Darwin Harbour or other Australian ports, containment would have been much more difficult, perhaps impossible, depending on the extent of its spread when detected. But "chance favours only the prepared mind" (Pasteur), and we were fortunate that the Northern Territory Government was experienced in the rapid and vigorous eradication of terrestrial pests and was able to transfer that experience to the marine environment.

In preparation for future events, the Aquatic Pest Management (APM) unit was established within the Northern Territory Government. APM completed the 12 month monitoring programme for *Mytilopsis* sp. and developed and implemented protocols that will reduce the risk of a second establishment of an invasive alien marine species in Northern Territory waters.

Consultation with local stakeholders recognised the four Darwin marinas as high-risk areas, and visiting international vessels as high-risk vectors. Since the eradication, all international vessels wishing to enter Darwin marinas are now inspected and treated prior to being issued clearance certificates. Entry to the marinas is prohibited without a clearance certificate.

Between May 1999 and June 2001, a total of 437 vessels including 364 yachts, 38 commercial fishing trawlers and 35 apprehended illegal vessels have been inspected by APM. The 35 apprehended vessels were identified as a high-risk category of vessels following the finding of significant black striped mussel fouling on the hulls of apprehended vessels moored within Darwin Harbour at a quarantine area during an APM monthly survey of high traffic areas in Darwin Harbour. Apprehended vessels typically travel from Southeast Asia, which is renowned for its biological diversity, including several undesirable marine species such as the black striped mussel and Asian green mussel. New protocols have been developed for apprehended vessels, and the Australian vessels that come into contact with them, to reduce the chance of them being moored close onshore before inspection. A plan to scuttle fouled apprehended vessels at sea, in areas where the risk of larvae reaching the coast are minimal, is under consideration in light of legal obligations.

The vessel inspection programme has intercepted at least four undesirable taxa: a variety of bryozoans (not identified to species), and three molluscs: *Musculista senhousia*, *Perna viridis* and *Mytilopsis* sp. However, monthly photographs of designated underwater surfaces and concurrent monthly checking of settlement collectors for the appearance of exotic fouling organisms have determined that the high traffic areas of Darwin Harbour, its marinas and Gove Harbour remain free of alien marine species that are known to be invasive.

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