

# Anthropogenic modification of estuaries: disturbance and artificial structures influence marine invasions

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Anthropogenic modification of estuaries: disturbance and artificial  
structures influence marine invasions



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Thesis submitted in fulfilment of the requirements for the degree of Doctor of  
Philosophy within the University of New South Wales

July 2009

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Estuarine environments are threatened by the synergistic effects of anthropogenic disturbance and bioinvasion. The construction of artificial structures (such as pilings and pontoons) provides a habitat resource in close proximity to vessel hulls that may be carrying a wide range of non-indigenous fouling species. In addition, the release and accumulation of toxicants from antifouling (AF) paints on vessel hulls creates a chemical disturbance that may reduce the invasion resistance of native communities. This thesis examines how shipping-related disturbances affect sessile communities, and in particular what role AF paints and artificial structures play in the invasion of estuaries.

Using a series of field-based experiments, I found that copper and tributyltin have the potential to influence both the transport of species to a new region, via the application of AF paints on vessel hulls, and their subsequent establishment, via the accumulation of AF biocides in estuaries. Temperature, pH, salinity and turbidity were also related to species distributions. During subtidal surveys of artificial and natural structures I found more non-indigenous species (NIS) on pilings and pontoons than on rocky reef, and shallow floating structures were identified as hotspots for invaders. These findings suggest that artificial structures play an important role in the initial establishment of sessile NIS in new regions. A restricted subset of NIS were also present on the reefs sampled during the survey and I conducted manipulative experiments to determine factors affecting the invasibility of turf and canopy-forming algal assemblages. The resident assemblage provided a barrier to most invaders, particularly when light and sedimentation levels were also high (i.e. on horizontal substrate). My results suggest that the areas of reef most susceptible to invasion are vertical rock walls and those subjected to frequent disturbances that release space.

In summary, this is the first study to relate copper and tributyltin contamination in the field to NIS distributions. My research has also highlighted the potential role of artificial structures in facilitating the establishment of NIS in estuaries and identified invasion threats to rock wall communities and disturbed reefs in estuarine systems.

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## **Abstract**

Estuarine environments are threatened by the synergistic effects of anthropogenic disturbance and bioinvasion. The construction of artificial structures (such as pilings and pontoons) provides a habitat resource in close proximity to vessel hulls that may be carrying a wide range of non-indigenous fouling species. In addition, the release and accumulation of toxicants from antifouling (AF) paints on vessel hulls creates a chemical disturbance that may reduce the invasion resistance of native communities. This thesis examines how shipping-related disturbances affect sessile communities, and in particular what role AF paints and artificial structures play in the invasion of estuaries.

Using a series of field-based experiments, I found that copper and tributyltin have the potential to influence both the transport of species to a new region, via the application of AF paints on vessel hulls, and their subsequent establishment, via the accumulation of AF biocides in estuaries. Temperature, pH, salinity and turbidity were also related to species distributions. During subtidal surveys of artificial and natural structures I found more non-indigenous species (NIS) on pilings and pontoons than on rocky reef, and shallow floating structures were identified as hotspots for invaders. These findings suggest that artificial structures play an important role in the initial establishment of sessile non-indigenous species in new regions. A subset of NIS were also present on the reefs sampled during the survey and I conducted manipulative experiments to determine factors affecting the invasibility of turf and canopy-forming algal assemblages. The resident assemblage provided a barrier to most invaders, particularly when light and sedimentation levels were also high (i.e. on horizontal substrate). My

results suggest that the areas of reef most susceptible to invasion are vertical rock walls and those subjected to disturbances that release space.

In summary, this is the first study to relate copper and tributyltin contamination in the field to NIS distributions. My research has also highlighted the potential role of artificial structures in facilitating the establishment of NIS in estuaries and identified invasion threats to rock wall communities and disturbed reefs in estuarine systems.

## **Chapter 1**

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### **General introduction**

The introduction of non-indigenous species (NIS) is posited as the biggest threat to biodiversity after habitat loss (Lubchenco et al. 1991, Vitousek et al. 1997, Wilcove et al. 1998, Carlton 2001, MEA 2005). For successful invasion to occur, a species must be transported outside of its native range (donor region) and then establish a self-sustaining population within the new area (recipient region). Impacts of NIS on native populations may then result from predation, competition, habitat alteration and/or the introduction of novel diseases and parasites (Gray 1997, Stachowicz et al. 2002a, Lewis et al. 2003).

Different selection filters act on non-indigenous species at different stages of the invasion process. At the transport stage, NIS are selected for a wide environmental tolerance as they must survive entrainment and subsequent changes in environmental conditions from the donor to recipient region (Johnston et al. 2009). This results in only a subset of invaders arriving in a new location and successful establishment will then depend on them finding a niche within the new community (Tilman 1997). As a consequence, a subset of transported species is actually able to establish and persist. This has also been defined in statistical terms as the “tens” rule (Williamson and Fitter 1996) and refers to the probability that one in every ten species transported is likely to arrive at a new destination, and one in ten of those arrivals will establish in the new region. The final “ten” refers to the statistical chance that an established species will become a pest. It should be acknowledged that this is a useful conceptual framework and not a strict rule, and the actual proportion of species surviving at each stage can and does vary (Jeschke and Strayer 2005, Lockwood et al. 2007). While we have some idea

of the selection agents acting on species introductions (Williamson and Fitter 1996), there are still agents have not been thoroughly identified.

Prior to the development of human transport networks, species would have expanded their ranges by natural dispersal. More recent cases of species dispersal have largely resulted from accidental or deliberate transport by humans (Carlton 1989, Ruiz et al. 2000). Patterns of marine invasion have closely followed an expansion in worldwide shipping, with changing trade routes increasing the number of both donor and recipient regions (Ruiz et al. 2000, Gollasch 2002, Minchin and Gollasch 2003). Vessels are travelling further, faster and more frequently, creating new opportunities for the introduction of NIS, and exerting even stronger selective pressures on the species being transported. Organisms are subjected to extremes of drag and antifouling (AF) biocides (on vessel hulls), salinity and dissolved oxygen (in ballast water) and temperature changes as the vessel moves between regions. Individuals remaining viable and attached after an international journey may display broad tolerance to a wide range of environmental conditions.

Upon arrival in a new environment, the establishment and dispersal of a NIS will depend on features such as growth and reproductive rates and the ability of the organism to evade native predators and/or parasites (Byers 2002b). The receiving community also has a major role to play in either facilitating invasion or providing a barrier to invading species. Invasibility is generally considered to be greatest in disturbed environments, and least in high diversity communities where resources are limited and all available niches may be occupied (Elton 1958, Davis et al. 2000, Levine 2000, Stachowicz et al. 2002a, but see Stohlgren et al. 2003, Fridley et al. 2007).

## **Disturbance and invasion**

Disturbance plays a major role in structuring communities. Direct effects of disturbance include damage or mortality in a community that results in the release of resources (Dayton 1971). Disturbance can also indirectly affect a community by altering competitive interactions and allowing the persistence of species that would otherwise be excluded (Dayton 1971, Dial and Roughgarden 1998). Community effects are dependent on the intensity and scale of the disturbance (Coffin and Lauenroth 1988, Maarel 1993, McCabe and Gotelli 2000), as well as the type of disturbance (Schratzberger and Warwick 1999, Cheroske et al. 2000). For example, the consumption of prey species by predators is likely to be highly specific and patchy, whereas damage from a storm event may be more indiscriminate and would generally extend over a larger scale.

Disturbances often result in the release of space, which is a major limiting resource in many terrestrial grassland and marine hard substrate communities (Davis et al. 2000, Stachowicz et al. 2002a). Recovery from these disturbances occurs when local species are able to recolonise the bare space. Recolonisation can be by native or non-indigenous species depending on the available propagules at the time and location of disturbance. Many NIS behave opportunistically; they are good colonisers, but poor competitors (Grime 1977, Burke and Grime 1996), and when disturbances occur at the same time as arriving propagules, it becomes more likely that they will be able to exploit the conditions (Clark and Johnston 2005, Britton-Simmons and Abbott 2008). In this way, disturbance can facilitate the establishment of non-indigenous species within an otherwise competitively superior native community.

Estuaries are highly susceptible to marine invasion because of the continuous supply of invasive propagules on transport vectors (e.g. vessel hulls) and the level of anthropogenic modification. Examples of estuarine disturbances include dredging, the addition of artificial structures and pollution (Gray 1997, Glasby and Connell 1999, Birch 2000). These have the potential to directly affect invasion by making the environment more suitable for invasion (Bulleri and Airoidi 2005). They can also indirectly aid invasion by reducing native species diversity and/or abundance and thus free up resources for invading species to exploit (Glasby et al. 2007, Piola and Johnston 2008a).

#### *Antifouling paints as agents of disturbance*

Antifouling paints are used to prevent the recruitment of fouling assemblages and until recently, tributyltin (TBT) was the most widely used primary biocide. The application of TBT on vessels < 25m (mainly recreational yachts) was banned in most developed countries in the late 1980s (Champ 2000) when its harmful effects on marine molluscs became apparent. Since this period, copper along with several ‘booster’ biocides has been the main AF replacement on all vessels (Srinivasan and Swain 2007). In November 2001 the International Maritime Organisation (IMO) adopted the “AFS Convention”, to ban the application of TBT on all vessels after 1 January 2003 and require its absence as an active coating on all vessels after 1 January 2008 (IMO 2001). The AFS Convention did not become internationally binding until September 2008, twelve months after its ratification by the required 25 states representing at least 25% of the gross tonnage of the world’s merchant shipping.

Antifouling biocides have the potential to affect fouling assemblages in two ways. On a vessel's hull they exert selection pressures for the recruitment and transport of organisms with heavy metal tolerance (Allen 1953, Floerl et al. 2004). The accumulation of biocides while vessels are in port also affects the water quality in receiving environments. Elevated copper concentrations found in marinas have been linked to recreational vessel traffic (Claisse and Alzieu 1993, Schiff et al. 2004), and high TBT levels have been found in embayments with many commercial vessels (Lenihan et al. 1990, Andersen 2004) as well as in pristine regions (Negri et al. 2004). Heavy metal pollution in ports and harbours has the potential to reduce the resilience of the resident native species and create opportunities for invading species (Piola and Johnston 2008a), but no data currently exists linking metal contamination in the field to NIS distributions.

*Artificial structures as agents of disturbance*

Artificial structures (e.g. pilings, pontoons and seawalls) are constructed in marine environments to support recreational and commercial shipping activities and can also increase the hard substrate habitat available for fouling species to settle and establish populations (Glasby et al. 2007). Such structures also provide a particular type of habitat, both moving and fixed, which invading species may be better able to exploit than native species (Dafforn et al. 2009b), and some evidence suggests that many NIS arriving on ship hulls are confined to artificial substrata (Paulay et al. 2002). However, where NIS have been able to invade natural systems they are associated with severe ecological consequences [e.g. *Membranipora membranacea* (Lambert et al. 1992) and *Caulerpa taxifolia* (Ceccherelli et al. 2002)]. The addition of artificial structures is hypothesised to increase the connectivity between hard substrates and create 'stepping



stones' that facilitate the establishment and dispersal of NIS in natural reef systems (Glasby and Connell 1999). However, to date there have been few studies comparing NIS on artificial and natural structures (but see Glasby et al. 2007) and we still have a limited understanding of the factors that determine whether an invader will establish in a natural system.

*Invasibility of natural reef*

Similarities can be drawn between studies of invasion in terrestrial grassland and marine sessile communities; space is the primary limiting resource in both systems (Stachowicz et al. 1999, Kennedy et al. 2002). However, grassland invaders are often functionally identical to the resident community, while on algal-dominated rocky reefs, invaders can be sessile invertebrates. Additionally, grassland communities generally form on horizontal substrate while marine sessile communities recruit to horizontal and vertical substrate, including overhangs. Algae and sessile invertebrates will compete for space, but are functionally very different e.g. a producer and consumer respectively. This could be an advantage to invading invertebrates if they are able to utilise a different niche, or could disadvantage them if the environmental conditions that promote algal growth actually exclude invertebrates (Sakai et al. 2001, Irving and Connell 2002).

Community ecology of natural rocky reefs suggests that native algae will dominate well-lit horizontal surfaces while native sessile invertebrates will be confined to shaded vertical surfaces or overhangs up to 180° (Baynes 1999, Miller and Etter 2008). Light promotes algal growth on horizontal reef surfaces and may result in low invasibility, but periodic disturbances that release space have the potential to open the community up to invasion (Sousa 1979, Lambert et al. 1992, Valentine and Johnson 2003, Dunstan and

Johnson 2004, Sanchez and Fernandez 2006, Britton-Simmons and Abbott 2008). High sediment levels on horizontal reef can also result in problems for sessile invertebrate invaders by smothering new recruits or clogging filter feeding apparatus (Ostroumov 2005), but macroalgae, particularly turfs, are highly resistant to sediment (Airolidi 1998, 2003).

The focus of this thesis has been hard-substrate assemblages, particularly non-indigenous species in several estuaries along the New South Wales coastline, Australia. Hard-substrate assemblages are a useful study system in which to examine the impacts of estuarine disturbances and artificial structures because they can be grown on settlement plates, and easily manipulated to test multiple experimental factors simultaneously. Specifically, I considered the ecological effects of copper and tributyltin and other physico-chemical variables on the estuarine distribution of NIS. I also investigated the role of artificial structures in the establishment and dispersal of NIS and the invasibility of native macroalgal communities.

### **Thesis outline**

To achieve the aims outlined above, I used a combination of observational and experimental techniques. I used a manipulative experiment to investigate the effect of copper and tributyltin (TBT) antifouling paints directly on the recruitment of invasive species as well as a large-scale survey to examine the effect of metal contamination in the field on the recruitment of invaders in estuaries (Chapter 2). In a review chapter I considered the environmental implications of past and current antifouling technologies with a particular focus on TBT and copper (Chapter 3). The addition of artificial structures such as wharf pilings and pontoons support vessel movements in and between

estuaries and I considered the effect of fixed (pilings) and moving (pontoons) structures on the recruitment of invaders (Chapter 4). I also compared NIS diversity between artificial and natural structures and investigated the factors contributing to invasion resistance in temperate rocky reefs (Chapter 5). This chapter describes the findings of several studies, including an in situ survey of NIS on artificial and natural structures and two manipulative field experiments examining the effects of patch size, resident assemblage, shading and orientation on marine invasion.

Chapter 2 – 5 of this thesis have been prepared in the form of stand-alone manuscripts for publication in peer-review journals. Chapter 2 and 4 have been published (Dafforn et al. 2009a, Dafforn et al. 2009b). As such, there may be some repetition between chapters. Reprints of published articles are included in the appendices at the end of the thesis.

## Chapter 2

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### **Links between estuarine condition and spatial distributions of marine invaders.**

#### **Abstract**

Non-indigenous species pose a significant threat to the environment and to global economies. Predictive and preventative measures are widely considered more effective in curtailing invasions than are eradication or control measures. Of key importance in the prediction of regional invasion risk are the environmental conditions that enable successful establishment. We surveyed native and non-indigenous sessile invertebrate diversity in each of two commercial (600 - 1500 vessels/yr) and two recreational estuaries (seven - nine marinas) in New South Wales, Australia. A nested hierarchical design was employed to investigate variation in sessile invertebrate diversity at the scales of site (1 – 3 km apart) and estuary (40 – 180 km apart). Settlement plates (15 x 15 cm) were used to sample invertebrates and background heavy metal loads were assessed using bioaccumulation in experimentally deployed oysters. Other physico-chemical variables were monitored monthly. Manipulative experiments were used to test the direct effects of exposure to copper and tributyltin (TBT) antifouling (AF) paints on sessile invertebrates. Native and non-indigenous species richness differed at various spatial scales, but showed no consistent difference between commercial and recreational estuaries. Instead, individual species distributions were strongly related to metal contamination, temperature, turbidity and pH. In experimental studies, several

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This chapter has been published (Appendix I):

Dafforn, K. A., T. M. Glasby, and E. L. Johnston (2009) Links between estuarine condition and spatial distributions of marine invaders. *Diversity and Distributions* **15**: 807-821.

species (mostly invaders) were more abundant on plates exposed to copper and/or TBT AF paints. We found higher levels of copper (and in some instances TBT) in recreational marinas than in commercial harbours. Our results demonstrate the importance of metal pollution and physico-chemical variables in the establishment of invaders in new regions. We have identified several native Australian species that have been exported overseas and suggested mechanisms contributing to their transport and establishment. Combining physico-chemical information about donor and recipient regions with species tolerances could go some way to predicting where future invasions may occur.

## **Introduction**

The introduction of non-indigenous species (NIS) is widely argued to be the second most important cause of native species decline after habitat loss (Vitousek et al. 1996). NIS have been associated with native species extinctions through predation, competition and habitat alteration (Mack et al. 2000). The reduction in native species abundances has associated economic costs, particularly when the species at risk is one of interest to farming or aquaculture. In the United States alone, the cost of management, outreach, control and eradication strategies for NIS across all ecosystems have been estimated in excess of \$138 billion per year (see reviews by Pimentel et al. 2000, Colautti et al. 2006). Recognition of the threat NIS pose to the environment and global economies has resulted in a push for predictive and preventative methods to control the spread of invaders (Mack et al. 2000, Hulme 2006, Keller et al. 2008). To effectively manage the invasion threat, managers need to be able to identify vulnerable areas where NIS will dominate (e.g. 'invasion hotspots' Holeck et al. 2004) and where they may act as a source of propagules to neighbouring regions.

Of key importance in the assessment of regional invasion risks are the environmental conditions that allow for establishment and dominance of species in a new region. The so-called ‘habitat suitability’ hypothesis suggests that successful introduction is more likely if species are matched with suitable environments (Williamson et al. 1986, Ribera and Boudouresque 1995, Blackburn and Duncan 2001, Miller et al. 2007). The invasion process is likely to select for species that have a wide environmental tolerance because they must survive the process of entrainment and transport (Carlton 2001, Johnston et al. 2009, Minchin et al. 2009). In the marine environment, ports and marinas are recognised as invasion hotspots (Carlton 1987, Hewitt et al. 2004, Hewitt and Campbell 2007) and transport vectors include the hulls of boats, ballast tanks or sea chests, and also the aquaculture industry (Allen 1953, Carlton 1985, Williamson et al. 1986, Ruiz et al. 2000, Coutts et al. 2003, Fofonoff et al. 2003, Minchin 2007). Conditions for invaders transported internally (ballast water) and externally (hull fouling) by maritime vessels can be highly stressful (Wonham et al. 2001, Minchin and Gollasch 2003). In addition to surviving high concentrations of heavy metals on the hulls and in the ballast tanks of vessels (Murphy et al. 2002, Finnie 2006, Hua and Liub 2007), marine invaders may be exposed to physical hardships during an oceanic voyage caused by the ship’s movement through the water and dramatic changes in the physico-chemical properties of the water body between geographical regions. Disturbances such as changes to temperature regimes and pollution in the recipient environment can also increase the susceptibility of a community to arriving invaders (Stachowicz et al. 2002b, Clark and Johnston 2005).

Ports and harbours are ideal systems within which to test theories about associations between invaders and environmental parameters. They are the first point of entry for NIS arriving on the hulls of vessels, in ballast tanks or sea chests (Carlton 1987), although since July 1 2001 discharge of “high risk” ballast water (including salt water from ports and coastal waters outside Australian territorial waters) is prohibited in Australian ports (AQIS 2008). Ports are also important foci of anthropogenic activities that can influence physico-chemical variables such as temperature, salinity, dissolved oxygen (DO), pH and turbidity. Changes in these variables may affect the growth and reproduction rates of resident species and alter their metabolic rates and feeding efficiencies (Salazar and Salazar 1996, Ostroumov 2005). Physico-chemical variables also have been found to influence the distribution of many marine species (Barry et al. 1995, Engle and Summers 1999, Akin et al. 2003, Roessig et al. 2004, Nicholson et al. 2008), and some studies have focused particularly on NIS. For example, Miller et al. (2007) found that the ability to withstand low salinity was an important predictor of molluscan invader success. Similarly, in the brackish waters of Europe, NIS were found to exploit environmental conditions that did not favour native species (Paavola et al. 2005). Levels of DO have also been found to affect interactions between native and non-indigenous species (Byers 2000, Jewett et al. 2005).

Heavy metal contaminants in estuaries can also influence the distribution of marine species and have been well studied in benthic communities (Brown et al. 2000, Morrissey et al. 2003), but comparatively little remains known about their effect on sessile invertebrate fouling communities (marine organisms that grow on hard substrates) (but see Moran and Grant 1993). Pollution research in Port Kembla Harbour, Australia found that fouling species showed differential responses to polluted

conditions with some native and non-indigenous species (e.g. *Galeolaria caespitosa* and *Watersipora arcuata*) unaffected by pollution and vice versa (e.g. *Bugula neritina*) (Moran and Grant 1993). The build-up of heavy metals in estuaries has been posited as a major driver of invasion outcomes through the provision of a competitive advantage to more tolerant NIS (Piola and Johnston 2008a). In fact transplant experiments found that pollution-tolerant fouling species relocated to unpolluted sites had a low survival rate and were quickly replaced by local, less tolerant species (Moran and Grant 1991). However, there are no data directly linking pollution levels and invasive marine species' distributions in the field. Levels of pollution will differ with respect to the primary activities in an estuary and will differ spatially and temporally within an estuary. For example, estuaries dominated by industry are likely to have high levels of contaminants from factory waste (particularly metals) and may accumulate high concentrations of tributyltin (TBT) from antifouling (AF) paints used on commercial vessels (e.g. oil tankers and coal transporters) (Lewis 2001, Lewis et al. 2004). In addition, periodic disturbances from activities such as dredging may release contaminants resulting in physiological and toxic stress to local species (Moran and Grant 1993). Estuaries surrounded by residential housing and occupied by recreational marinas are likely to have lower levels of heavy metals, although copper from the AF paints used on recreational vessels has been found to accumulate in the water around marinas (Claisse and Alzieu 1993, Floerl and Inglis 2003, Schiff et al. 2004, Warnken et al. 2004).

Antifouling paints are applied to the external submerged surface of maritime vessels and often contain heavy metals to prevent the settlement of fouling species. Since the 1980s, most recreational vessels (< 25 m long) in developed countries have been banned from using TBT-based AF paints (Champ 2000). These recreational vessels have generally



reverted to copper-based AF paints, while commercial vessels continued to use TBT [although use of TBT has been gradually phased out since 2003 and banned since January 2008 by countries ratifying the International Convention on the Control of Harmful Antifouling Systems on Ships (IMO 2001)]. Based on the predicted half-life of TBT and associated compounds, many recreational estuaries should now harbour only very low levels of this contaminant in the water column (Champ and Seligman 1996).

This study examined the prediction that commercial and recreational estuaries support distinct populations of non-indigenous and native fouling species, with more NIS in commercial ports. It is proposed that these patterns of fouling species' distribution are related to different numbers and types of transport vectors in the two types of estuaries and to the different contaminants and physico-chemical conditions present in each. We also examined the response of non-indigenous and native fouling species to two copper-based and one TBT-based AF paint to test the hypothesis that the presence of copper AF paints would favour marine invaders. Results are interpreted with reference to species that are non-indigenous to Australia and species that are native to Australia, but have been introduced overseas; the latter are termed 'exported' species (ES) (Dafforn et al. 2009b). Hereafter, NIS and ES are referred to as 'invaders'. It is important to consider invaders as a specific group including not only NIS, but also native species that pose a risk of export to other regions.

## **Methods**

### *Study sites*

Experiments were conducted in two commercial and two recreational estuaries along the coast of New South Wales, Australia, between June 2006 and February 2007 (Fig.

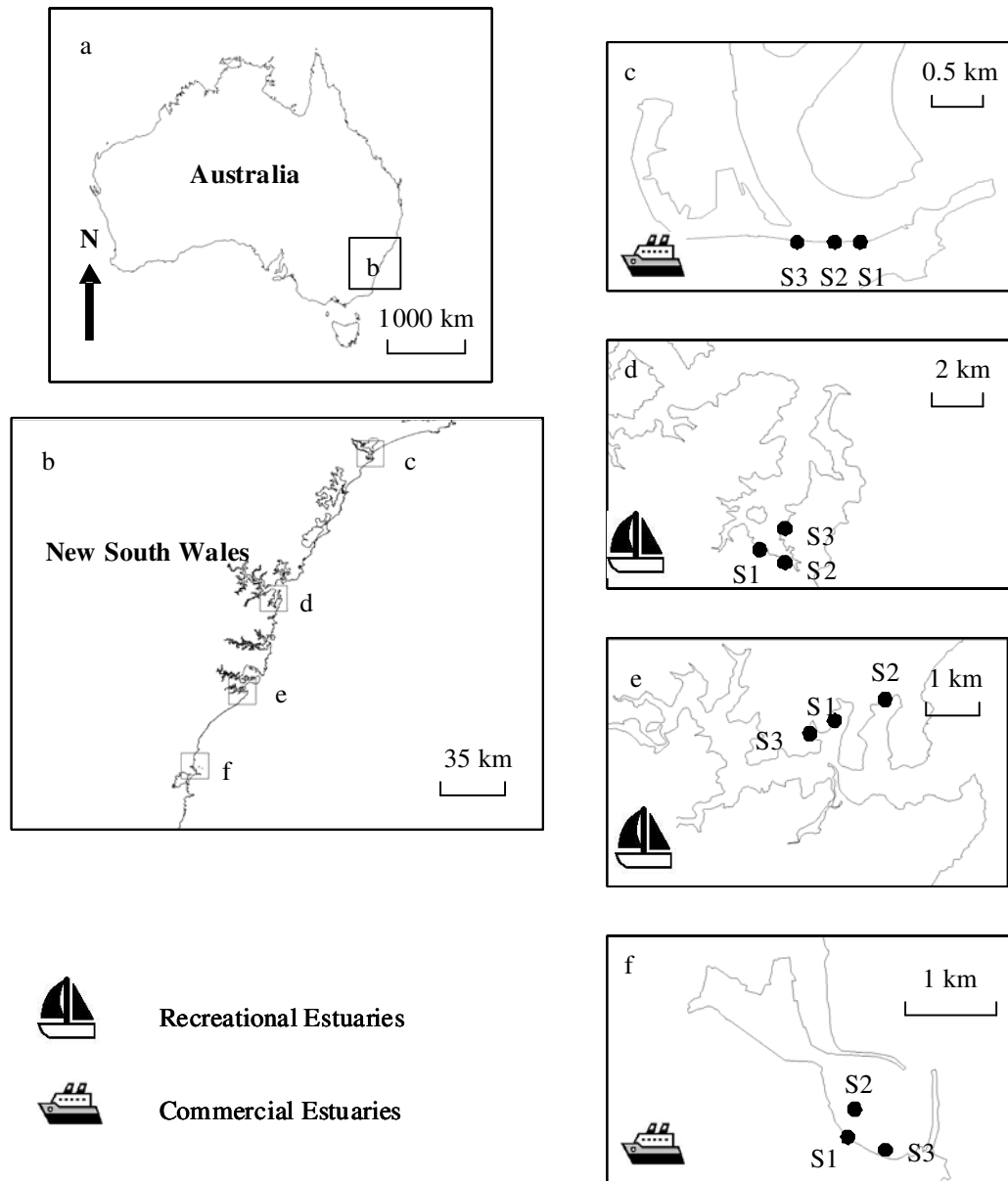
2.1). Port Kembla and Newcastle Harbour are commercial estuaries receiving international vessel traffic. Port Kembla receives *c.* 600 vessels/year (PKPC 2006) and Newcastle Harbour 1500 vessels/year (NPC 2006). Both harbours have a single small recreational marina located > 500 m away from the main port area, but each harbour is dominated by commercial facilities. Pittwater and Port Hacking are exclusively recreational estuaries and neither supports commercial shipping activities. Pittwater has nine marinas and two sailing clubs and Port Hacking has seven marinas and hundreds of private pontoons and moorings accommodating an estimated 50 - 200 boats at each marina (Dafforn, pers. obs.). A spatially nested hierarchical design was employed to investigate variation in species recruitment within these estuaries. Three sites (*c.* 1 – 3 km apart) were sampled in each estuary. Each recreational site was situated at a marina, and each commercial site was situated at a docking terminal (Fig. 2.1).

*Biodiversity survey and experimental comparison of antifouling paints*

In order to sample sessile invertebrate (fouling) assemblages in these estuaries six settlement plates (controls) (15 x 15 cm) were deployed at each site at a depth of 2 m below mean low water springs (MLWS (the estuaries have tidal ranges of between 1 and 2 m). Settlement plates were made of black Perspex (3.5 mm thick) and were roughly sanded. Control plates were attached to plastic frames using two cable ties in the outer 3 cm and were spaced ~20 cm apart on the frame. Frames were suspended vertically and were hung in shaded places, either under floating pontoons (fixed depth) or on fixed pilings (variable depth) beneath jetties *c.* 2 - 3 m from the sea floor at MLSW. To test hypotheses about effects of AF paints on sessile invertebrate assemblages, an additional 18 settlement plates were treated with one of three different paints and simultaneously deployed at each site; six were treated with a 3 cm border of

‘Micron Extra’ (copper diuron, CuDi), six with ‘Intersmooth 360’ (copper zinc pyrithione, CuZnP) and six with ‘Superyacht 800’ (TBT). CuDi is commonly used by recreational vessels, while CuZnP is only available to commercial vessel owners and has been introduced as an alternative to TBT. Control plates (described above) were treated with a 3 cm border of non-toxic primer to allow direct comparisons with the paint treatments. Settlement plates were deployed on three frames with two replicates of each AF treatment on each frame (eight plates per frame) spaced ~20 cm apart. Frames were separated by 3 - 5 m within each site.

Jetties and pontoons are the dominant structures in commercial and recreational estuaries and therefore, for logistic reasons and to reflect the primary substrate available in those areas, it was necessary to attach frames to stationary structures (jetties) in commercial estuaries and to moving structures (pontoons) in recreational estuaries. Research by Holloway and Connell (2002) found differences in assemblage development between stationary and moving structures; however they compared moving plates that were either partially above the surface or submerged on the water line, and did not test effects at greater depths. To investigate whether such structural differences may have confounded our comparison of assemblages between estuary types, we deployed 11 x 11 cm Perspex settlement plates attached to larger frames that were moving or stationary at 0.5 m or 2 m depth for a period of three months (Dafforn et al. 2009b). Invaders (NIS + ES) were more numerous on moving than stationary structures at 0.5 m, however they showed no difference between moving and stationary structures at 2 m (Dafforn et al. 2009b), which was the depth at which plates were deployed in the current study. Similarly, when species were analysed individually, there was no difference between stationary or moving structures at 2 m for several of



**Figure 2.1.** Sampling locations of berths and marinas (c) Newcastle Harbour containing S1 = Dyke 1, S2 = Dyke 2 East and S3 = Dyke 2 West, (d) Pittwater containing S1 = The Quays Marina, S2 = RPAYC and S3 = Heron Cove Marina, (e) Port Hacking containing S1 = Burraneer Bay Marina, S2 = Cronulla Marina and S3 = Dolans Bay Marina and (f) Port Kembla containing S1 = Jetty 4 South, S2 = Jetty 4 North and S3 = Jetty 6.

the species that were dominant in the current study (Dafforn et al. 2009b). In addition, patterns for other dominant species were generally in the opposite direction to those found between commercial and recreational estuaries. For example, some invaders have been found to be more abundant on moving plates at depths of 2 – 3 m, including *Diplosoma listerianum*, *Pyura stolonifera*, *Amphibalanus variegatus* and *Bugula neritina* (Glasby 2001, Dafforn et al. 2009b). In the present study, these species occupied more space in commercial estuaries, where the frames were always attached to stationary structures. As such, our comparison of communities between commercial and recreational estuaries may have failed to detect differences between estuary types, for some species, because the sampling in recreational estuaries was biased towards some invaders and as such potentially overestimated their abundance in recreational estuaries.

### *Data analyses*

After eight months, settlement plates were retrieved, photographed and preserved in 5% formaldehyde. Images were used to estimate percentage covers of taxa using one hundred randomly arranged points placed over the central 9 x 9 cm area. Organisms were identified to species level where possible and identities were confirmed by examination of preserved plates. Species that could be identified were classified as native, non-indigenous or exported. Those that could not be identified or whose origins were uncertain were classified as cryptogenic (Details in Appendix II).

Data for total non-indigenous, native (including exported) and cryptogenic species richness (number of species) and percentage cover on control plates were compared between estuary types using a three-factor nested analysis of variance (ANOVA).

‘Estuary Type’ was treated as a fixed orthogonal factor and ‘Estuaries (within Estuary Type)’ and ‘Sites (within Estuaries)’ were random, nested factors. All data were assessed for normality using residual frequency histograms and for homogeneity of variance using Cochran’s C-test. We then tested for a correlation between non-indigenous and native species richness and results are presented graphically. Percentage covers of dominant species (> 5%) on control plates were also compared between estuary types using a three-factor nested ANOVA (as described above) and the main results presented in-text (full ANOVA tables are included in Appendix III).

To investigate the effects of AF paints, we compared results of species’ patterns across all experimental units to test whether the patterns of difference among control and AF treatments occurred more frequently than would be expected by chance (Binomial test, Underwood 1997). AF treatments were analysed separately for each estuary, and frames were the replicates in these analyses (n = 18 in each estuary type).

*Comparison of heavy metals and physico-chemical variables between estuaries*

To test whether levels of heavy metals differed between commercial and recreational estuaries, the accumulation of metals was measured in experimentally deployed oysters. Oysters (and settlement plates) were spaced appropriately to prevent cross-contamination from the different AF paints. Dafforn et al. (2008) took water samples from directly next to settlement plates in an attempt to measure the release of copper and TBT from the painted borders and found that levels were below detectable limits (< 5 µg/L copper and < 2 ng/L TBT). Three mesh bags of 10 oysters were suspended at 2 m depth at each field site for 12 weeks, and then collected and depurated for 48 h in containers of filtered sea water before storage at -10°C (Robinson et al. 2005). Each

replicate (n = 3 per site) consisted of a composite of four oysters selected randomly from a single mesh bag, freeze dried and ground to powder following Hardiman and Pearson (1995). Sub-samples of 0.4 g of freeze-dried oyster powder were added to 5 ml of distilled HNO<sub>3</sub>, 2 ml of H<sub>2</sub>O<sub>2</sub>, and 3 ml of Milli-Q water in digestion vessels and microwave digested at 190°C for 20 min. After digestion, samples were made up to 30 ml using Milli-Q water and analysed using ICP-MS at the Solid State and Elemental Analysis Unit (UNSW, Sydney). Each sample was analysed for Al, As, Cd, Co, Cu, Hg, Ni, Mn, Pb, Sn and Zn. Recoveries were generally within 90 - 100% of expected values (NIST 1566b Oyster Tissue). Where recoveries were outside this range, the metals data were omitted from analysis (Al, Hg, Ni, Pb and Sn). Because of costs of TBT analysis, three replicates were analysed per estuary (one per site) by the National Measurement Institute (Sydney).

To test for differences in physico-chemical variables between sites, temperature, salinity, turbidity, pH and oxygen levels were measured during the study using a portable water profiler (Yeo-Kal Model 611, Yeo-Kal Electronics, Sydney). Three replicate measurements were taken monthly at each site for eight months from June 2006 to February 2007 and the profiler was deployed at the same depth as the experimental frames (2 m). Data collected from the heavy metal analysis and physico-chemical variables were analysed with a three-factor nested ANOVA (see above) using a mean of all months taken for each site.

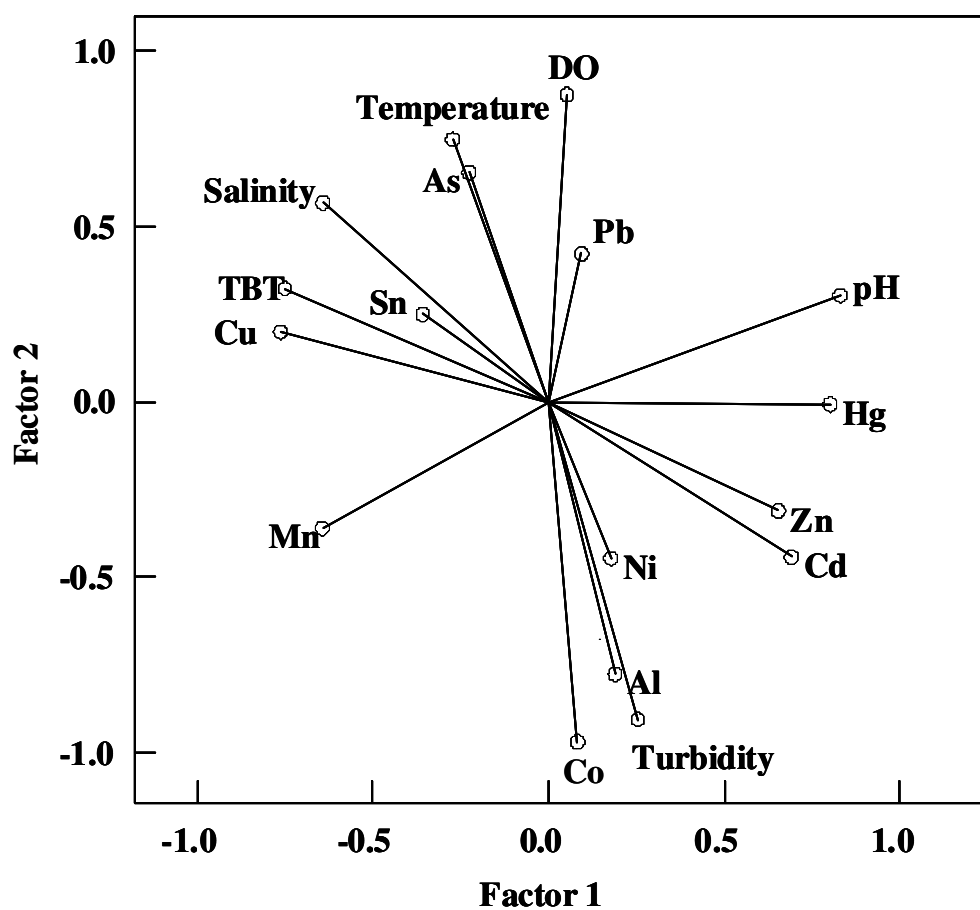
Principal Components Analysis was performed on the data collected for heavy metals and other physico-chemical variables in each of the estuaries (Fig. 2.2). Data were untransformed and subject to a varimax rotation to maximize the sum of the variances

of the loading factors, resulting in a reduced number of factors contributing to the variances (Kaiser 1958). Factors 1 and 2 explained 60% of the variance. Where several variables were highly correlated ( $r > 0.7$ ), the biologically redundant variable was omitted from regression analysis (Quinn and Keough 2002). For example, where levels of a particular heavy metal were consistently below mean natural 'background' concentrations (values from Scanes and Roach 1999) found in oysters, and therefore unlikely to have a biological impact, they were omitted (As, Cd, Co, Mn; Table 2.1). To interpret the degree of heavy metal contamination at the different sites, levels of copper in our experimentally deployed oysters were compared with natural 'background' levels in uncontaminated oysters (Scanes and Roach 1999), and levels of TBT with oyster shell deformity thresholds (Batley et al. 1992) and presented graphically. We then performed regression analyses on the remaining variables and percentage cover of the dominant fouling species. SPSS was used to fit a line that best represented the data; in some cases this was a curve. Regressions are presented graphically for  $r^2 \geq 0.3$ .

## Results

Sixty taxa were identified during the study (most to genus or species) and of these, 17 were classified as native (eight of these have been recorded as non-indigenous overseas and are therefore considered ES) and 25 as non-indigenous. The remaining 18 were classified as cryptogenic. The fauna included species of encrusting and arborescent bryozoans, barnacles, solitary and colonial ascidians, and serpulid polychaetes. The dominant taxa (those with an average percent cover of  $> 5\%$ ) were used in univariate analyses and included the NIS: *Hydroides elegans* (Haswell, 1884), *Bugula neritina* (Linnaeus, 1758), *Watersipora subtorquata* (d'Orbigny, 1842), *Styela plicata* (Lesueur, 1823), *Botrylloides leachi* (Savigny, 1816) and *Diplosoma listerianum* (Milne-Edwards,





**Figure 2.2.** Principal Components Analysis using data collected for heavy metals and other physico-chemical variables in each of the estuaries.

**Table 2.1.** Mean metal concentrations ( $\mu\text{g/g}$  dry weight) found in oysters deployed at 3 sites in 4 estuaries. Oyster replicates were lost from S2\* in Port Hacking and were replaced with the mean value of S1 and S3 from the same estuary.

Estuary	Site	As	Cd	Co	Cu	Mn	Zn	TBT (ng/g)
<i>Observed concentrations</i>								
Newcastle	S1	0.91	0.15	0.052	16.54	1.53	<b>470.05</b>	13
Newcastle	S2	1.25	0.11	0.048	11.12	2.26	<b>351.63</b>	12
Newcastle	S3	0.97	0.15	0.060	<b>25.40</b>	1.74	<b>528.74</b>	16
Port Kembla	S1	1.20	0.14	0.030	<b>40.54</b>	1.39	<b>494.96</b>	<b>150</b>
Port Kembla	S2	1.19	0.13	0.032	<b>40.71</b>	1.92	<b>419.23</b>	15
Port Kembla	S3	1.08	0.14	0.037	<b>101.20</b>	1.82	<b>531.06</b>	<b>120</b>
Port Hacking	S1	1.39	0.11	0.040	<b>54.79</b>	1.26	<b>474.44</b>	25
Port Hacking	S2*	1.41	0.11	0.038	<b>57.62</b>	1.36	<b>474.96</b>	44.5
Port Hacking	S3	1.42	0.11	0.036	<b>60.45</b>	1.45	<b>475.49</b>	64
Pittwater	S1	1.41	0.09	0.042	<b>88.48</b>	2.24	<b>437.93</b>	<b>86</b>
Pittwater	S2	1.11	0.10	0.045	<b>128.19</b>	2.40	<b>360.61</b>	<b>190</b>
Pittwater	S3	1.28	0.08	0.042	<b>126.76</b>	1.95	<b>421.34</b>	<b>110</b>
<i>Background concentrations</i>								
Mean		1.88	0.54	0.064	21.6	2.53	277	(Batley, Scammell et al. 1992) 84, 88, 107, 112 ng/g – shell deformities present

**Bold** indicates values above natural “background” concentrations or above levels known to cause shell deformities.

1841), the ES: *Amphibalanus variegatus* (Darwin, 1854), *Balanus trigonus* (Darwin, 1854), *Celleporaria nodulosa* (Busk, 1881) and *Pyura stolonifera* (Heller, 1878) and the native species: *Salmacina australis* (Haswell, 1884) (Table 2.2).

*Spatial variation in species distribution and diversity*

Non-indigenous, native and cryptogenic species richness and percent cover did not differ significantly between estuary types (Fig. 2.3). Instead, we found the strongest differences in species recruitment to be between individual estuaries and/or among sites rather than between commercial and recreational estuaries. Native species dominated space at nine out of the 12 sites (Fig. 2.3), and this native dominance of space can be attributed primarily to *Amphibalanus variegatus* in Newcastle (40 - 55%), *Salmacina australis* in Port Kembla and Port Hacking (10 - 35%) and *Celleporaria nodulosa* in Pittwater (10 - 50%). Numbers of native and non-indigenous species on each frame were strongly positively correlated ( $r = 0.693$ ; Fig. 2.4), ranging from  $2.5 (\pm 0.4)$  to  $7.1 (\pm 0.7)$  for natives and  $2.3 (\pm 0.3)$  to  $7.2 (\pm 0.9)$  for non-indigenous species (Fig. 2.4).

The exported barnacles, *Amphibalanus variegatus* and *Balanus trigonus* were the major space occupiers in the study and their percentage covers varied between individual estuaries ( $F_{2,8} = 69.31$ ,  $p = 0.000$  and  $F_{2,8} = 10.38$ ,  $p = 0.006$  respectively), but not between estuary types (i.e. commercial vs. recreational). The non-indigenous serpulid polychaete, *Hydroides elegans* occupied more space on plates deployed in one commercial port (Port Kembla) than another (Newcastle) ( $F_{2,8} = 28.55$ ,  $p = 0.000$ ). Percentage cover of *H. elegans* also differed significantly between the two recreational estuaries, while the native serpulid, *Salmacina australis* varied in its abundance among sites within one of the recreational estuaries (Port Hacking; SNK,  $p < 0.05$ ). Percentage

covers of the non-indigenous bryozoans, *Bugula neritina* and *Watersipora subtorquata* and the exported bryozoan, *C. nodulosa* varied among sites within estuaries ( $F_{8,60} = 3.42$ ,  $p = 0.003$ ;  $F_{8,60} = 3.62$ ,  $p = 0.002$  and  $F_{8,60} = 3.96$ ,  $p = 0.000$  respectively). *W. subtorquata* was generally absent from all sites apart from in Port Hacking where percentage cover was between 10 and 20%. Percentage cover of the exported ascidian, *Pyura stolonifera* and the non-indigenous ascidian, *Botrylloides leachi* varied significantly between the two recreational estuaries ( $F_{2,8} = 5.68$ ,  $p = 0.005$  and  $F_{2,8} = 7.05$ ,  $p = 0.017$  respectively), but not between commercial estuaries. In contrast, the non-indigenous solitary ascidian, *Styela plicata* showed variation in its recruitment among sites in Port Kembla ( $F_{8,60} = 8.68$ ,  $p = 0.000$ ). The colonial ascidian, *Diplosoma listerianum*, was the only NIS to differ significantly between estuary type and occupied more space on plates in commercial than in recreational estuaries ( $F_{1,2} = 33.75$ ,  $p = 0.028$ ).

#### *Heavy metals in oyster tissue*

Heavy metal levels were also highly variable among sites but did not differ significantly between commercial and recreational estuaries. Levels of Cu (in all but two commercial sites;  $F_{8,24} = 3.28$ ,  $p = 0.011$ ; Fig. 2.5) and Zn (at all sites;  $F_{8,24} = 0.471$ ,  $p = 0.864$ ) were found to be well above natural 'background' concentrations (Table 2.1). At site 3 in Port Kembla (commercial) and all sites in Pittwater (recreational), Cu levels were three times greater (88 – 128  $\mu\text{g/g}$ ) than 'natural' background concentrations (21.6  $\mu\text{g/g}$ ) (Fig. 2.5). Zn levels were almost double (350 - 530  $\mu\text{g/g}$ ) the levels that would be expected in uncontaminated oyster tissue (277  $\mu\text{g/g}$ ) at all sites in the study (Table 2.1). TBT levels were highest in Pittwater and Port Kembla ( $F_{2,8} = 4.85$ ,  $p = 0.042$ ) and were

at levels that could potentially cause shell deformities (Table 2.1; Fig. 2.5), although no deformities were observed in the experimental oysters.

*Relationships between species percent cover and heavy metal loads*

Six species were strongly related to heavy metal loads, including five invaders and one native species. Percentage cover of the exported barnacle, *Amphibalanus variegatus* was negatively related to levels of Cu and TBT and showed a weak positive relationship with Zn (Table 2.2 a, b, c; Fig. 2.6 a, b). *A. variegatus* also responded positively to the TBT paint treatment, but only at commercial sites (Table 2.2 d). Percentage cover of *Balanus trigonus* (exported) was not related to background levels of Cu, TBT or Zn (Table 2.2 a, b, c), and was reduced on all the AF treatments in commercial estuaries (Table 2.2 d).

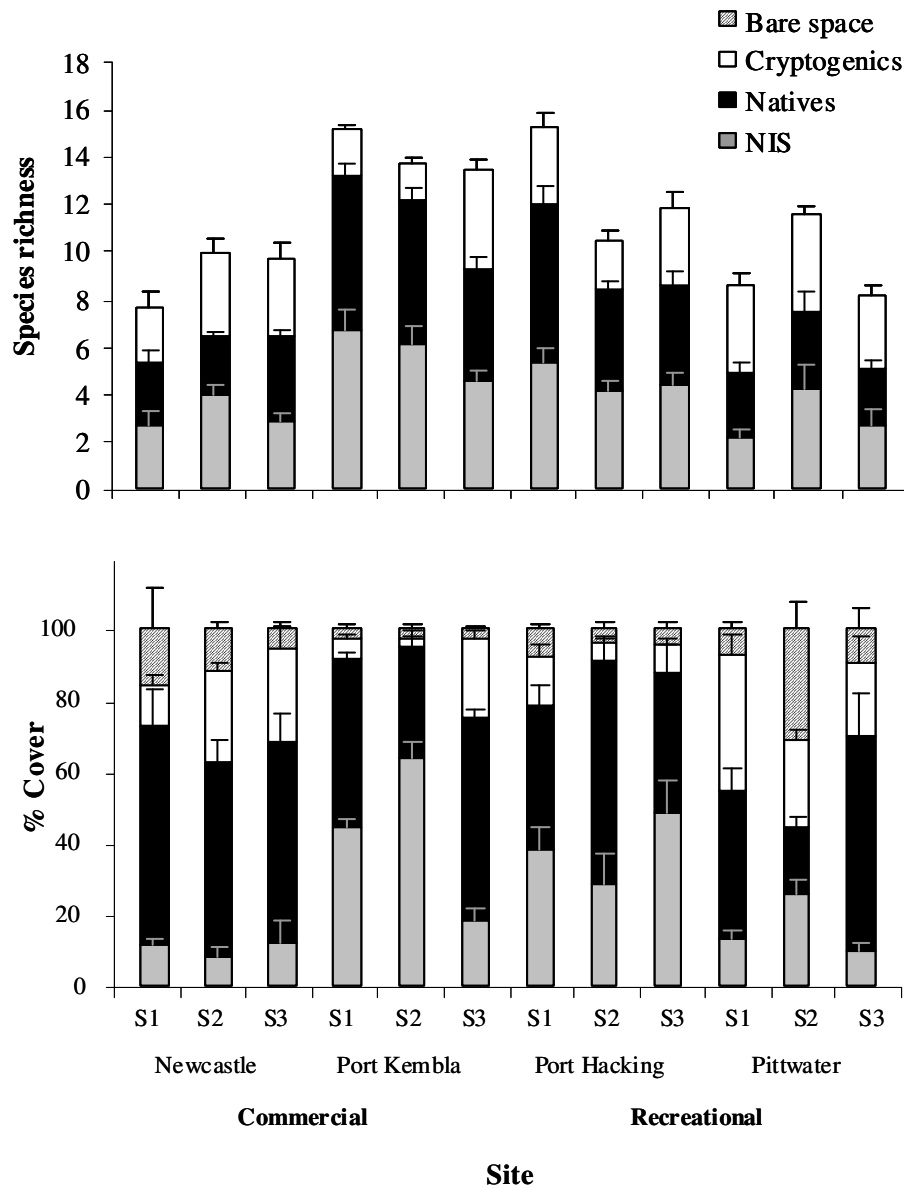
The serpulids *Hydroides elegans* (non-indigenous) and *Salmacina australis* (native) showed similar patterns in their percentage covers between estuaries and sites, and these were positively related to background levels of Cu (both species) and TBT (*H. elegans* only) (Table 2.2 a, b; Fig. 2.6 c, d). *S. australis* and *H. elegans* also showed a positive response to experimentally applied CuZnP, CuDi (*S. australis*) or TBT paints (*H. elegans*) (Table 2.2 e), but only at the sites where background Cu/TBT levels were already elevated (generally recreational sites at Pittwater).

**Table 2.2.** (a-c) Regression analyses ( $r^2$  values) of species percent cover and heavy metal loads. (d-e) Summary of binomial tests showing patterns among AF treatments which occurred significantly more ( $p < 0.05$ ; pattern occurred on 13 frames out of 18) than would be expected by chance in commercial and recreational estuaries. Species are classified as native (N) non-indigenous (NIS) and exported (ES; i.e. native to Australia, but listed as invasive in other parts of the world).

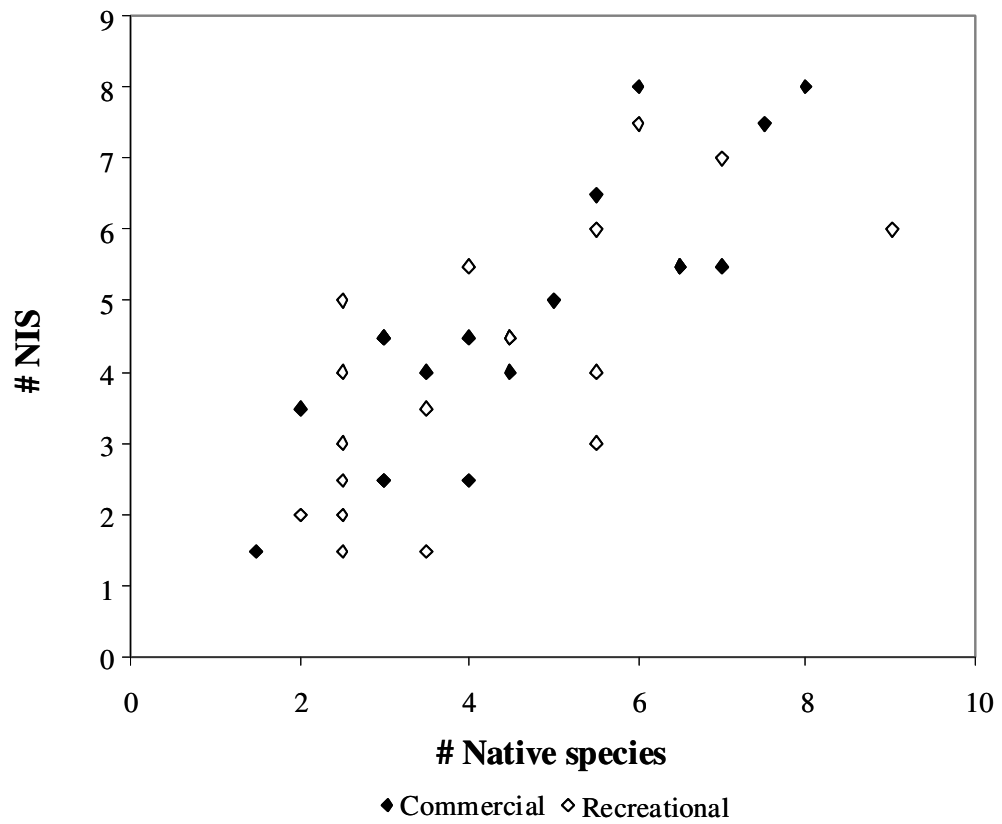
Classification	Species	(a) Cu	(b) TBT	(c) Zn	(d) Commercial estuaries			(e) Recreational estuaries		
					CuDi	CuZnP	TBT	CuDi	CuZnP	TBT
ES (Otani et al. 2007)	<i>Amphibalanus variegatus</i>	(-) 0.603 <sup>^</sup>	(-) 0.531 <sup>^</sup>	0.151	—	—	TBT > Ctrl <sup>***</sup>	—	—	—
ES (Otani et al. 2007)	<i>Balanus trigonus</i>	-	-	-	Ctrl > CuDi	Ctrl > CuZnP	Ctrl > TBT	—	—	—
NIS (Hutchings et al. 1989)	<i>Hydroides elegans</i>	0.494 <sup>^</sup>	0.447 <sup>^</sup>	-	—	—	—	—	—	—
N (Haswell 1884)	<i>Salmacina australis</i>	0.177	-	-	—	—	—	CuDi > Ctrl <sup>*</sup>	CuZnP > Ctrl <sup>*</sup>	TBT > Ctrl <sup>*</sup>
NIS (Keough and Ross 1999)	<i>Bugula neritina</i>	(-) 0.357 <sup>^</sup>	(-) 0.713 <sup>^</sup>	-	—	—	—	—	—	—
NIS (Keough and Ross 1999)	<i>Watersipora subtorquata</i>	-	-	-	CuDi > Ctrl	—	—	—	—	—
ES (Ingilis et al. 2006a, b)	<i>Celleporaria nodulosa</i>	0.275 <sup>^</sup>	-	-	—	—	—	—	Ctrl > CuZnP <sup>***</sup>	Ctrl > TBT
ES (Castilla et al. 2004)	<i>Pyura stolonifera</i>	(-) 0.258 <sup>^</sup>	-	-	—	—	Ctrl > TBT	—	—	—
NIS (Hewitt et al. 2004)	<i>Borysthenoides leachi</i>	-	-	-	—	—	—	—	—	—
NIS (Keough and Ross 1999)	<i>Syella plicata</i>	-	-	-	—	—	—	—	—	—
NIS (Hewitt et al. 2004)	<i>Diplosoma listerianum</i>	-	-	-	—	—	—	—	—	—

(a-c) **Bold** indicates where these regressions represent a significant relationship between the variables ( $p < 0.05$ , <sup>^</sup> =  $p < 0.01$ ). — indicates no significant patterns. (-) indicates relationship between species percent cover and heavy metal loads was negative.

(d-e) \* indicates response to paint treatment similar to relationship with background metal levels; \*\* indicates response to paint treatment opposite to relationship with background metal levels.

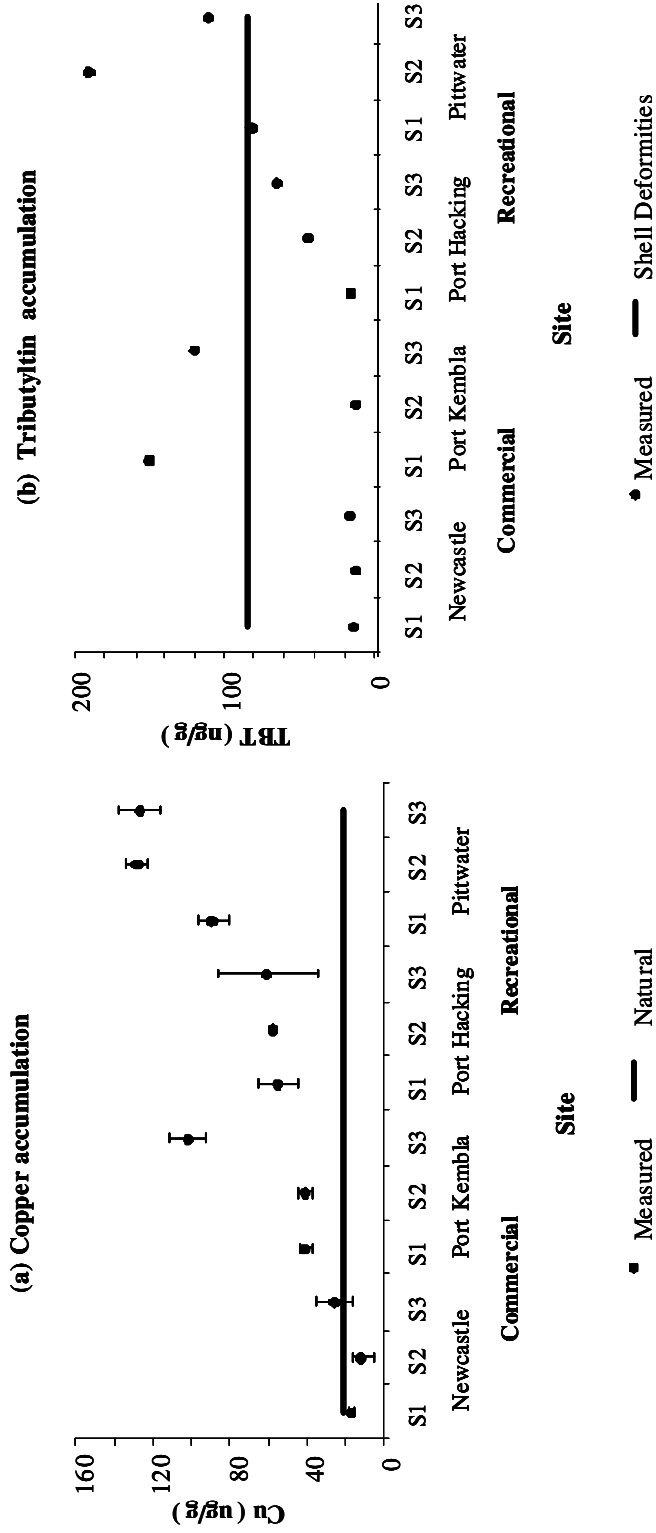


**Figure 2.3.** Species richness and percent covers (+ S.E.) of non-indigenous, native and cryptogenic species (and bare space) collected on settlement plates deployed at 3 sites within estuaries of commercial or recreational vessel activity.



**Figure 2.4.** Correlation plot of the number of non-indigenous and native species (richness) on each frame of three frames at three sites in each recreational or commercial estuary.





**Figure 2.5.** Comparison of copper ( $\mu\text{g/g}$  dry weight) and tributyltin ( $\text{ng/g}$  dry weight) concentration in oysters experimentally deployed at 3 sites within estuaries of commercial or recreational activity. 3 replicates per site are reported for (a) copper accumulation and 1 replicate per site for (b) tributyltin accumulation.

The non-indigenous bryozoan, *Bugula neritina* was more patchily distributed and its percentage cover was negatively related to levels of Cu and TBT (Table 2.2 a, b; Fig. 2.6 e, f), but showed no response to the AF treatments (Table 2.2 d, e). In contrast, percentage cover of the non-indigenous bryozoan, *Watersipora subtorquata* was not related to background Cu or TBT levels (Table 2.2 a, b), but did show an effect of the AF treatments. Specifically, the percent cover of *W. subtorquata* increased on CuDi plates compared with control plates (significant at commercial sites; Table 2.2 d). Percentage cover of the exported *Celleporaria nodulosa* was positively related to increasing background Cu levels (Table 2.2 a) and the bryozoan responded negatively to the CuZnP and TBT paint treatments (Table 2.2 e).

The exported ascidian, *Pyura stolonifera* was less abundant at sites with high background Cu levels (Table 2.2 a) and its percentage cover was negatively affected by TBT on plates at most commercial sites, but not at recreational sites (Table 2.2 d, e). The non-indigenous ascidians, *Botrylloides leachi*, *Styela plicata* and *Diplosoma listerianum* showed no relationship with background heavy metal loads and no effect of the AF treatments.

#### *Relationships between species percent cover and physico-chemical variables*

Nine species were strongly related to changes in physico-chemical variables, including eight invaders and one native species. Of these variables, DO and salinity varied between estuaries ( $F_{2,8} = 46.55$ ,  $p = 0.000$  and  $F_{2,8} = 16.03$ ,  $p = 0.002$  respectively). Temperature, turbidity and pH varied between sites within estuaries ( $F_{8,24} = 31.54$ ,  $p = 0.000$ ;  $F_{8,24} = 5.38$ ,  $p = 0.001$  and  $F_{8,24} = 13.50$ ,  $p = 0.000$  respectively). The barnacle, *Amphibalanus variegatus* was negatively related to DO, salinity and temperature, and

positively related to turbidity (Table 2.3; Fig. 2.7 a, b, c) while percentage cover of *Balanus trigonus* increased with increasing DO, temperature and pH (Table 2.3; Fig. 2.7 d, e). Percentage cover of *Hydroides elegans* was positively related to increasing salinity (Table 2.3; Fig. 2.7 f), and increased percentage cover of *H. elegans* and *Salmacina australis* was also related to increased temperatures (Table 2.3; Fig. 2.7 g). *H. elegans* and *S. australis* were negatively related to pH and turbidity respectively (Table 2.3). Percentage covers of the bryozoans, *Bugula neritina* and *Watersipora subtorquata* were also related to turbidity; positively and negatively respectively (Table 2.3; Fig. 2.7 h) and *Celleporaria nodulosa* was negatively related to pH (Table 2.3; Fig. 2.7 i). The solitary ascidian, *Pyura stolonifera* was not related to the measured physico-chemical variables while the colonial ascidian *Botrylloides leachi* was positively correlated with pH (Table 2.3; Fig. 2.7 j). Percentage cover of *Styela plicata* also increased with increasing DO and pH and decreasing turbidity (Table 2.3; Fig. 2.7 k, l).

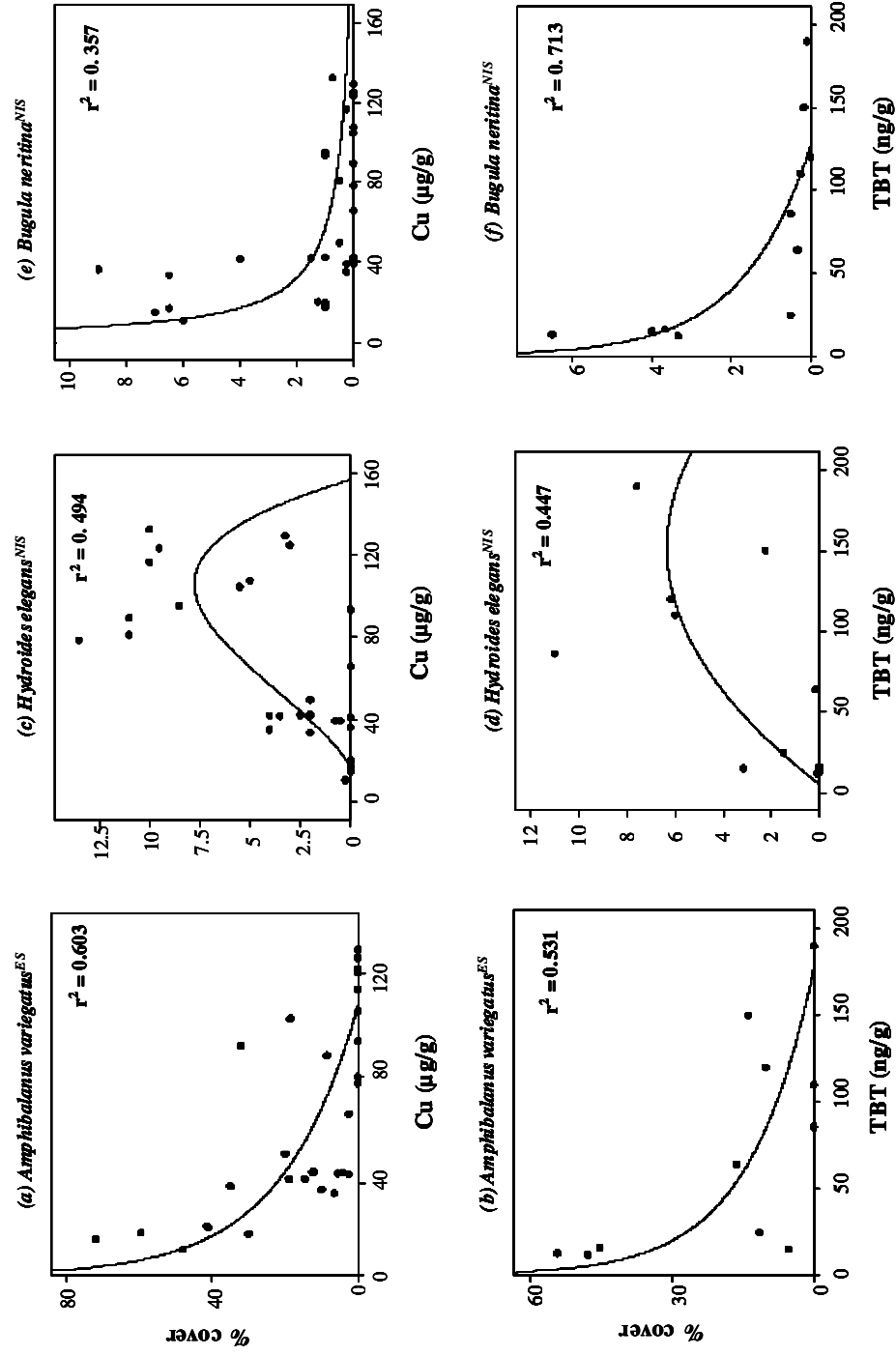
## Discussion

Estuaries are among the most highly disturbed marine environments receiving large inputs of contaminants and high volumes of shipping traffic, and as such provide an ideal environment for invasive species to establish and persist (Carlton 1996b, Piola and Johnston 2008a). We compared recruitment of sessile invertebrates in four impacted estuaries (recreational or commercial vessel activity) with differing contaminant loads and physico-chemical conditions, as well as the effect of different AF paint treatments on recruitment. This study has highlighted the potential role of metal pollution and physico-chemical variables in the establishment of invasive fouling species in new regions. We found that levels of anthropogenic impacts (in the form of heavy metal

**Table 2.3.** Regression analyses ( $r^2$  values) of species percent cover and physico-chemical variables.

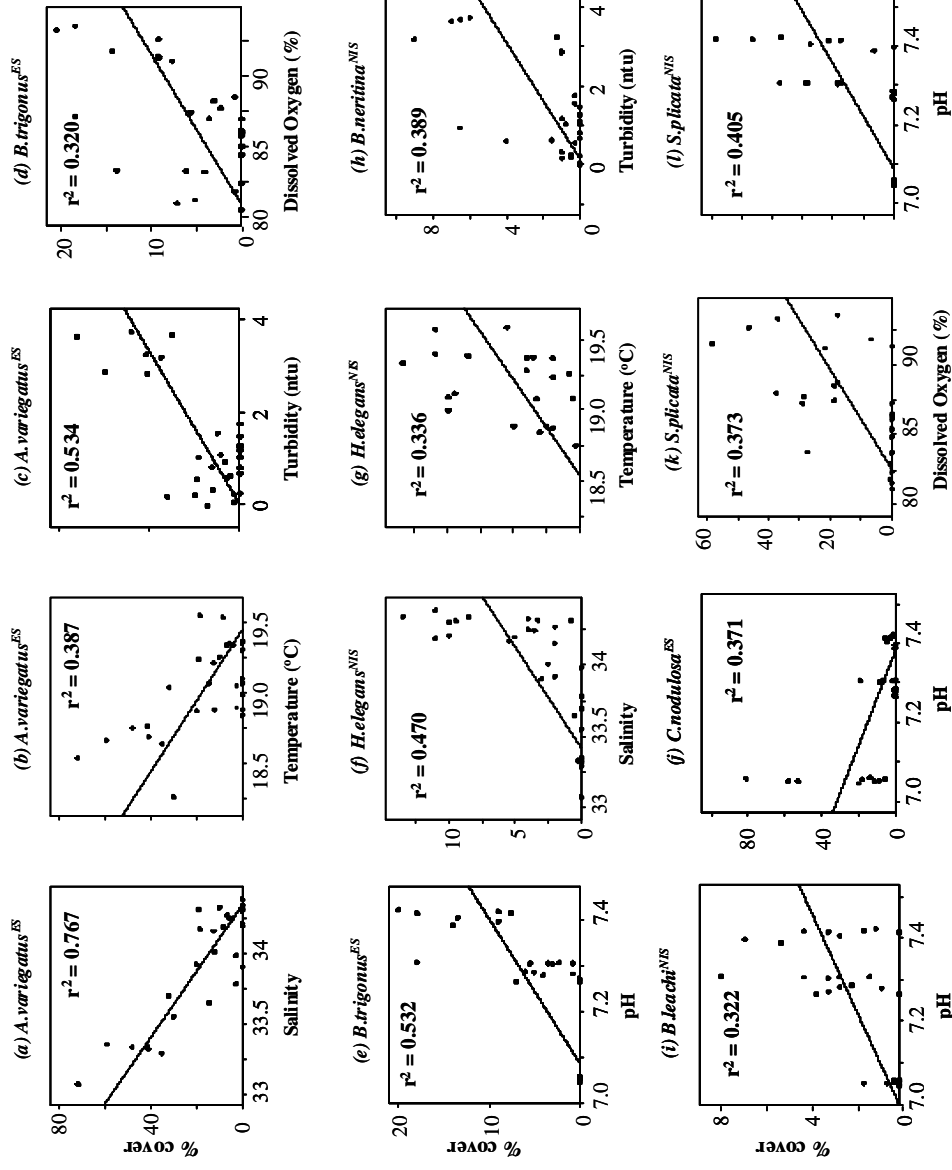
Species	(a) Dissolved Oxygen (%)	(b) Salinity	(c) Temperature (°C)	(d) Turbidity (ntu)	(e) pH
<i>Amphibalanus variegatus</i>	(-) <b>0.285</b> <sup>^</sup>	(-) <b>0.767</b> <sup>^</sup>	(-) <b>0.387</b> <sup>^</sup>	<b>0.534</b> <sup>^</sup>	-
<i>Balanus trigonus</i>	<b>0.320</b> <sup>^</sup>	-	<b>0.162</b>	-	<b>0.532</b> <sup>^</sup>
<i>Hydroides elegans</i>	-	<b>0.470</b> <sup>^</sup>	<b>0.336</b> <sup>^</sup>	-	(-) <b>0.297</b> <sup>^</sup>
<i>Salmacina australis</i>	-	-	<b>0.204</b>	(-) <b>0.141</b>	-
<i>Bugula neritina</i>	-	(-) <b>0.268</b> <sup>^</sup>	(-) <b>0.207</b>	<b>0.389</b> <sup>^</sup>	-
<i>Watersipora subtorquata</i>	-	-	-	(-) <b>0.218</b>	-
<i>Celleporaria nodulosa</i>	-	-	-	-	(-) <b>0.370</b> <sup>^</sup>
<i>Pyura stolonifera</i>	-	-	-	-	-
<i>Botrylloides leachi</i>	-	-	-	-	<b>0.322</b> <sup>^</sup>
<i>Styela plicata</i>	<b>0.373</b> <sup>^</sup>	-	-	(-) <b>0.275</b> <sup>^</sup>	<b>0.405</b> <sup>^</sup>
<i>Diplosoma listerianum</i>	-	-	-	-	-

**Bold** indicates where these regressions represent a significant relationship between the variables ( $p < 0.05$ , <sup>^</sup> =  $p < 0.01$ ). “-” indicates no significant patterns.



**Figure 2.6.** Regression analyses of species percent cover and heavy metal loads. Results are presented for  $r^2 > 0.3$ .

## 2. Invader distribution in estuaries



**Figure 2.7.** Regression analyses of species percent cover and physico-chemical variables. Results are presented for  $r^2 > 0.3$ .

contamination) and natural physico-chemical variables were more important correlates of species distributions (including 10 invaders and one native species) than the dominant estuary type (recreational versus commercial). This has implications for the management of vectors as well as ports and marinas which may act as sources of propagules for invasive species.

Non-indigenous species can be more abundant in estuaries than on open coasts (Wasson et al. 2005) and have also been found in estuaries that lack commercial shipping (e.g. Wasson et al. 2005, Wyatt et al. 2005). Cohen et al. (2005) sampled several different habitats within port and non-port areas and found no difference in NIS richness between them; however, their results could have been influenced by the rapid assessment methodology e.g. search effort and taxonomic skills (Campbell 2008) and the likelihood of different substrate types (Glasby 2000), ages of assemblages (Glasby 1999a) and sample sizes at each location. Our study controlled the age of assemblage and substrate composition, but substrata in commercial estuaries were stationary while those in recreational estuaries were moving. We found that the dominant taxa differed in their percentage covers between estuaries and sites, but this was not related to estuary type (i.e. recreational versus commercial), except for the non-indigenous ascidian *Diplosoma listerianum* which was more prevalent in commercial estuaries. Recreational estuaries had just as many NIS as commercial estuaries in spite of the differences in international vessel traffic, a similar result to Wyatt et al (2005). Native species were found to dominate space in nine of the 12 sites. Numbers of non-indigenous and native species were strongly positively correlated with each other, which agree with findings from other large-scale experimental studies of invasion (Lonsdale 1999, Davis et al. 2000, Levine 2000, Stohlgren et al. 2003). Our comparison between commercial and

recreational estuaries may not have detected differences for some species because of the structures available for our experimental plates. The invertebrate larvae of many fouling species are known to disperse short distances from the adult populations (Keough 1983) and therefore the relationships between species abundances and metals or physico-chemical variables, which occurred at smaller spatial scales (between sites, 1 – 3 km) may be more important considerations in the development of fouling assemblages.

We found levels of copper and zinc to be well above ‘natural’ background levels in 10 of 12 sites (Scanes and Roach 1999). Tributyltin contamination was also found to be a substantial problem in large recreational marinas (with levels that could potentially result in shell deformities in oysters) despite a ban on its application on boats < 25 m since 1989 and recent studies suggesting that TBT contamination is no longer a problem in port areas (Dowson et al. 1993, Evans et al. 1995, but see Gibson and Wilson 2003). TBT chemistry is largely dependent on local environmental conditions and its partitioning between dissolved and particle-adsorbed states depends on factors such as particle concentration and organic carbon content, salinity and pH (Harris et al. 1996). While TBT has a half-life of a few days in the water column (Seligman et al. 1996) in sediments it is thought to vary considerably both spatially and temporally, potentially in the order of months to years (Harris et al. 1996). In highly turbid areas, the TBT uptake potential of sediments is increased (Harris et al. 1996), which may partially explain why TBT levels were lower in the water column in the well-flushed and highly turbid waters of the commercial Newcastle Harbour than in the more sheltered recreational marinas (Floerl and Inglis 2003).



Heavy metals have long been recognised as important selection agents acting on aquatic organisms (reviewed by Klerks and Weis 1987). In polluted areas, organisms are under selective pressure for increased resistance to toxicants. This can result in physiological acclimation as tolerance is gained through exposure to sublethal concentrations, or the evolution of genetically based resistance through natural selection (Klerks and Weis 1987, Levinton et al. 2003). Several physiological mechanisms are recognised for coping with metal stress including the release of extracellular metabolites which bind to metals and reduce the metal concentration surrounding the organism (Fogg and Westlake 1955, McKnight and Morel 1979, Fisher and Fabris 1982). Some invertebrates also exhibit sequestering mechanisms such as the binding of metals to inducible metallothioneins (Olafson et al. 1979, Suzuki et al. 1980, Thompson et al. 1982, Engel and Brouwer 1986, Jenkins and Sanders 1986, Roesijadi 1986) or in granules and vesicles (Brown 1977, George and Pirie 1979, Lowe and Moore 1979, Mason et al. 1984).

The NIS *Hydroides elegans* responded positively to the copper AF treatments and dominated in areas where background copper levels were high. *H. elegans* is generally thought of as a copper-tolerant species (Allen 1953, Johnston and Keough 2003, Dafforn et al. 2008, Piola and Johnston 2008a), and exhibits tolerance of TBT (this study) which together may have aided its cosmopolitan dispersal on vessel hulls (Pettengill et al. 2007) and establishment in polluted harbours around the world since the 1800s (Ruiz et al. 2000). We have also demonstrated the potential for several native species to thrive in conditions of elevated copper (*Salmacina australis* and *Celleporaria nodulosa*), (but see Dafforn et al. 2008, Piola and Johnston 2008a) or TBT (*Salmacina australis*). Five native species also responded positively to the anti-fouling paints

(*Amphibalanus variegatus* and *S. australis*) or exhibited a tolerance (*Balanus trigonus* and *Pyura stolonifera* – recreational estuaries and *C. nodulosa* – commercial estuaries and CuDi in recreational estuaries). Metal tolerance therefore has the potential to advantage some native species within both donor and recipient ports and harbours that have elevated metal levels and a less tolerant resident biota, thereby increasing their chances of being exported overseas on hulls painted with copper- or TBT-based AF paint. With the exception of *S. australis*, these are all exported species with introduced ranges of Japan [*A. variegatus*, *Balanus trigonus*; (Otani et al. 2007)], New Zealand [*C. nodulosa*; (Inglis et al. 2006b, a)] and Chile [*P. stolonifera*; (Castilla et al. 2004)]. Japan, along with China, USA, South Korea and New Zealand are the major destinations for Australian maritime trade (BTRE 2007). The high (and increasing) volume of trade between these areas represents a significant risk of invasion from Australian species that have been exported. Interestingly, *S. australis* is recognised as endemic to Australia, but its high tolerance to copper, particularly in recreational estuaries suggests its potential for export around the globe. Combining information about shipping routes with information about species metal tolerances could enhance predictions about potential exports to other regions.

Physico-chemical variables including pH, temperature and turbidity were also important predictors of the distribution of fouling species and in three instances, explained > 50 % of the variation in species percent cover. Percentage cover of *A. variegatus* increased with increasing turbidity while cover of the solitary ascidian, *Styela plicata*, decreased. Many ascidians are negatively affected by suspended sediment. For example, the solitary ascidians, *Ciona intestinalis* and *Ascididiella aspersa*, are sensitive to inorganic material in the water column which reduces their filter feeding efficiency and can lead

to reduced growth rates and mortality (Robbins 1985). Excessive sediment can also result in burial and clogging of ascidian siphons and branchial structures (Bakus 1968). Potentially the increased sediment load in the water column inhibits growth of ascidian recruits, causing mortality and reducing competition for space with other taxa such as *A. variegatus*. Indirect benefits of reduced ascidian densities have been recorded previously for barnacles, bryozoans and serpulids (Johnston and Keough 2003).

pH was also strongly correlated with percentage cover of five invaders. Mean decreases in pH of 0.4 units were related to ~20% decreases in cover of *B. trigonus* and *S. plicata*. In the future, the increased sequestration of carbon dioxide by the ocean as a consequence of global warming is expected to lower pH levels with realistic changes in the order of 0.5 pH (RS 2005). This could have a direct effect on the physiology of marine organisms (particularly calcifying species e.g. barnacles). Increased ocean acidification may also have indirect effects on marine organisms by modifying the chemistry of toxins such as trace metals and increasing bioavailability (RS 2005). Similarly, predicted temperature increases of 1.8 – 4 °C by the year 2100 (IPCC 2007) could enhance toxic effects of metals (reviewed by Sokolova and Lannig 2008). This can partly be explained by increased metabolic activity that results in higher uptake, but also the bioavailability of metals increases at higher temperatures due to the increased solubility of metal compounds. Interestingly, several species that were correlated with copper and TBT, including *H. elegans*, *S. australis*, *A. variegatus* and *Bugula neritina*, showed a similar relationship with temperature, suggesting potential interactive effects of these physico-chemical variables. Future increases in metal bioavailability through ocean acidification and global warming may further advantage metal tolerant species

and result in their increased dominance of polluted areas where they can act as a propagule source for further export.

There is a widespread trend to reduce heavy metal contamination of estuaries and ports (Minchin and Gollasch 2003). TBT contamination has diminished (Evans et al. 1995), but copper, which is still commonly used in AF paints, is accumulating in estuaries and having toxic and sublethal effects on marine species (Claisse and Alzieu 1993, Hall Jr et al. 1998). Minchin and Gollasch (2003) suggested that remediation of harbours might lead to increased potential for NIS to invade and establish. We would predict the opposite based on this study and the work by Piola and Johnston (2008a), which suggest that reducing pollution loads in harbours could increase the resilience of native communities and reduce the dominance of invaders thereby reducing the number of invasive propagules available for export. One method for reducing metal loads in ports and harbours would involve legislation to enforce the use of non-toxic AF strategies. There currently exist several non-toxic alternatives to copper on the market and new technologies are in the process of being developed to replace harmful biocides in AF paints (Srinivasan and Swain 2007). However, if these alternatives prove less effective at preventing biofouling, then we risk increasing the transfer rate of NIS.

## **Conclusion**

Research points to the importance of the precautionary principle when dealing with NIS (Floerl et al. 2005), that is, every introduction should be considered potentially harmful. Past eradication attempts have proven costly and difficult (Willan et al. 2000, Anderson 2005, Coutts and Forrest 2007) and many current management programs aim to prevent the arrival of new pests. To this end, donor region and vector management are essential.

Our results suggest that recreational estuaries are just as likely to harbour invaders as commercial harbours subject to extensive international vessel operations (see also Wasson et al. 2001) and their dominance is often related to metal loads and the prevailing physico-chemical conditions. Of particular concern is how the effect of metal contamination might be magnified by a changing climate. To this end it is important for us to consider the potential for increased temperature and ocean acidification to increase metal bioavailability and thereby increase the vulnerability of ports and estuaries to invasion.

## **Chapter 3**

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### **Ecological impacts of past, current and future antifouling strategies.**

#### **Abstract**

Marine biofouling has both economic and environmental costs. Biofouling increases drag on vessels resulting in higher fuel consumption. Vessel hull fouling is also an important vector for the transport of non-indigenous species. Antifouling (AF) technologies incorporating heavy metals (e.g. copper and tributyltin (TBT)) have been developed to prevent settlement of organisms on vessels. Shipping activity currently accounts for 80% of total world trade and the widespread use of biocides in AF paints on vessels has introduced high levels of contamination into the environment and raised concerns about their toxic effects on marine communities. The recent ban on tributyltin (1 January 2008) and increasing regulation of copper AF paints have prompted development of non-toxic AF coatings. This review synthesises existing information regarding the ecological impact of AF biocides in a wide range of organisms and highlights directions for the management of AF paints. Future growth in seaborne trade appears likely and therefore an understanding of the role of AF paints as environmental stressors is critical. We focus particularly on representatives of the recent past (copper and TBT) and the present (copper and ‘booster’) biocides. We identify knowledge gaps within AF research and provide recommendations relating to the regulation and phasing-out of copper in AF paints.

#### **Introduction**

Marine biofouling describes the community of organisms that settle and grow on the external surfaces of submerged structures such as rocks and boulders or seaweeds and sessile invertebrates (WHOI 1952). Anthropogenic modification of the marine

environment has resulted in more opportunities for fouling organisms due to the abundance of hard surfaces on artificial structures such as pilings, pontoons, pipelines, cooling water intakes, aquaculture enclosures and vessel hulls (Visscher 1928, Maguire 1956, Glasby and Connell 1999). Within hours of a structure's submergence, a slime layer develops, which is comprised of microscopic organisms (bacteria and algae) bound within an extracellular matrix of polymeric substances (Zobell and Allen 1935, Steinberg et al. 2002). This layer promotes the settlement and attachment of macro-organisms, including larvae of invertebrates such as ascidians, serpulids and barnacles, by providing biochemical cues for settlement and increasing their adherence to the substrate (Zardus et al. 2008). The progression from micro- to macro-fouler occurs between days and weeks and often results in the development of a sessile assemblage comprising barnacles, ascidians, hydroids, bryozoans and algae (e.g. Maki and Mitchell 2002).

Biofouling is ubiquitous in the marine environment and is a major problem for the shipping industry (Fig. 3.1). Shipping accounts for approximately 80 % of world trade and seaborne trade has increased 3.1 % over the past 3 decades (ICS&ISF 2009). Growth of organisms on a vessel hull increases frictional drag which reduces ship speed or requires increased power and fuel consumption to maintain speed (Abbott et al. 2000). Slime films alone can impart powering penalties of 21%, with heavy calcareous biofouling increasing this penalty to 86% (Schultz 2007). Fuel costs account for up to 50% of operating costs and the initial development of a slime layer can increase fuel consumption by up to 1% (Champ and Pugh 1987). The economic costs of hull fouling have been a driving force behind the development of antifouling (AF) technologies, a global industry that is now worth approximately US\$ 4 billion annually (Wright 2009).

Antifouling measures include the use of coatings on vessel hulls to inhibit the settlement of marine organisms. These have traditionally incorporated toxicants including copper and tributyltin into a paint matrix that gradually leaches the biocide from the surface layer to prevent settlement (Champ and Pugh 1987). The widespread use of toxicants in AF paints has resulted in high levels of contamination in the environment and raised concerns about their effects on marine communities (Claisse and Alzieu 1993, Alzieu 2000, Antizar-Ladislao 2008, Thomas and Brooks 2010). Copper has a long history of association with AF paints while TBT coatings were developed only recently (1960s) and, after a relatively short period, abandoned (1990s on vessels < 25 m) (reviewed by Lewis 1998). Since 1 January 2008 regulations have been in place to prohibit the use of TBT in all AF paints (IMO 2001) due to the ecological and economic impacts of TBT contamination on oyster and whelk populations (Alzieu et al. 1986, Gibbs and Bryan 1986) and bioaccumulation in marine mammals and other oceanic species (Tanabe 1999, de Brito et al. 2002). This has resulted in a return to, and increased application of copper-based AF paints. These can also have toxic effects on aquatic organisms (Bryan 1971) and have been implicated in the transport and establishment of some metal-tolerant non-indigenous fouling species (Allen 1953, Floerl et al. 2004, Piola and Johnston 2008a, Piola et al. 2009). Economic and environmental impacts of AF biocides have resulted in a push for non-toxic alternatives (Clare 1998, Carson et al. 2009, Piola et al. 2009). Some non-toxic silicone-based coatings, known as foul release coatings have been developed (Townsin and Anderson 2009). These do not prevent settlement of fouling organisms, but reduce the attachment strength such that when the vessel is in motion any accumulated biofouling will slough off as drag increases. However, foul release coatings have restricted application as they are only self-clean



effectively on high speed/high activity vessels. Hence the development of a general use non-toxic AF paint is far from complete (Yebra et al. 2004).

In this paper we review the environmental impacts of AF strategies with reference to the history and regulation of AF technology. Past reviews have tended to focus on (a) economic impacts [e.g. TBT(Champ 2003)], (b) toxic effects of biocides [e.g. TBT (Hall Jr and Bushong 1996, Maguire 2000), copper (Bryan 1971, Flemming and Trevors 1989) and ‘boosters’ (Thomas 2001, Thomas and Brooks 2010)], (c) biological invasion [e.g. copper (Piola et al. 2009)] or (d) progress in developing non-toxic alternatives (Clare 1996, 1998). Consequently, due to these multiple but disparate foci, our general understanding of the ecological impacts of various AF strategies is somewhat fragmented (but see Lewis 1998). Here we integrate environmental effect information regarding each of the AF strategies and describe the most current AF regulations. Finally, we consider the future for AF strategies, including the implications of climate change for the use of heavy metals and the development of novel non-toxic AF coatings.

### **Antifouling technology: history and regulation**

Antifouling technology has developed in close association with increased maritime transportation of people and cargo. With greater frequency and distance of travel it became apparent to early mariners that their speed was substantially reduced by the accumulation of vessel hull fouling and that this resulted in increased journey times. The early Phoenicians (1500-300 BC) are credited with the first advance in AF technology in the form of lead and copper sheets to prevent biofouling on their wooden boats (WHOI 1952). By the 17<sup>th</sup> and 18<sup>th</sup> centuries copper along with arsenic and

mercury were being incorporated in coatings that could be applied to vessel hulls (WHOI 1952). Copper was an effective and widely used biocide, however some organisms were found to be tolerant and settled on hulls regardless of its presence (Allen 1953, Russell and Morris 1970). In addition, the effectiveness of copper in the AF paints was relatively short-lived so dry dockings of vessels for cleaning and paint reapplication were required frequently [c. every 18 months (Lewis 1998)].

#### *Tributyltin*

With the discovery of the antifouling efficacy of trialkyltins, hull fouling was thought to be a problem of the past (Minchin and Gollasch 2003). Tributyltin was first used in freshwater systems to eradicate molluscs harbouring the parasitic worm *Schistosoma* (Champ and Pugh 1987). The success of TBT as a molluscicide signalled its potential in marine systems and, from 1961, TBT was introduced as a biocide in AF paints (Champ and Pugh 1987). ‘Modern’ TBT paints were highly effective against barnacles and molluscs, two major problem groups for vessel operators (Allen 1953, Knight-Jones and Crisp 1953, Wisely 1963). Tributyltin was not as effective against the early fouling stages (‘slime layer’) as copper, which continued to be used during this period as a co-biocide (Nichols 1988). Initially, TBT was incorporated in conventional or ‘free association’ paints, which relied on passive leaching to deliver toxicant to the surface to inhibit growth (Nichols 1988). Conventional paints had a limited lifetime of around two years and were superseded in the 1970s by self-polishing copolymer (SPC) paints (lifetime > 5 years). In SPC paints the TBT copolymer provides both the biocide and paint matrix, and this hydrolyses in seawater to release the TBT leaving an unstable surface layer that gradually erodes to expose a fresh layer of active paint (Champ and Pugh 1987, Ten Hallers-Tjabbes 1997).

Authorities first became aware of the problems associated with TBT in the early 1980s, when several bays in France with highly productive oyster farms experienced major declines due to reduced oyster spatfall, anomalies in larval development, and shell malformation affecting from 80 to 100% of individual oysters (Alzieu et al. 1986). What had been hailed as a saviour for the maritime industry became an ecological disaster. The decline in reproduction and increase in shell deformities led to the 1982 ban on TBT use on vessels < 25 m in France (Alzieu et al. 1986). This ban targeted mainly recreational vessels as they spend long periods in high densities and were therefore perceived as the biggest contributor to TBT contamination from passive leaching (Alzieu et al. 1986). Imposex (the development of male characteristics on female gastropods) was soon after identified as another negative effect of TBT contamination (Bryan et al. 1986). Ecological impacts on gastropod populations due to imposex and major growth abnormalities in farmed oysters triggered subsequent TBT restrictions on small vessels in the UK (1987), USA (1988), Canada (1989), Australia (1989) and the EU (1989) (Champ 2000). This resulted in most recreational vessel owners in these countries reverting to copper-based substitutes while commercial vessels continued to use TBT, although the latter were commonly restricted to using low TBT release SPC systems. Since restrictions were introduced, recovery has been observed in some TBT-impacted populations (Rees et al. 2001, Morton 2009), however the ecological problems associated with TBT have persisted in some locations, notably in the vicinity of shipyards and commercial port areas (Gibson and Wilson 2003, Gibbs 2009).

In November 2001 the International Maritime Organisation (IMO) adopted the “AFS Convention”, to ban the application of TBT on all vessels after 1 January 2003 and require its absence as an active coating on all vessels after 1 January 2008 (IMO 2001). The AFS Convention did not become internationally binding until September 2008, twelve months after its ratification by the required 25 states representing at least 25% of the gross tonnage of the world’s merchant shipping. However, some anticipatory action was taken in some regions; for example, European Union Regulation (EC) No. 782/2003 banned the application of TBT-paints on all EU-flagged vessels from 1 January 2003 (Morton 2009) and in Australia the registration of all anti-fouling paints containing TBT was revoked in March 2003.

It is likely that TBT continues to be used on domestic vessels in non-signatory countries (Antizar-Ladislao 2008). For example, tributyltin remains unregulated in Bahrain, Egypt and Israel where studies have found high levels of TBT in seawater and sediment, and evidence of imposex (Hasan and Juma 1992, Rilov et al. 2000, Barakat et al. 2001).

### *Copper*

Even while TBT dominated the international marketplace, copper continued to be used, both in cheaper paints for small craft or as a co-biocide to boost performance of some TBT paints (Young et al. 1979, Nichols 1988). Since the ban on TBT, copper has once again become the predominant antifouling biocide, but its use in AF paints is under scrutiny in several countries and has an uncertain future. There are concerns about elevated copper concentrations around ports and marinas (Hall Jr and Anderson 1999, Jones and Bolam 2007, Dafforn et al. 2009a) that often exceed water quality guidelines and have the potential to create environmental problems (Claisse and Alzieu 1993,

Srinivasan and Swain 2007). AF paints are a significant source of copper through passive leaching, and from their removal and application during boat maintenance (Hall Jr et al. 1998), but it is often difficult to distinguish the relative inputs from different sources (e.g. industrial waste and urban runoff (but see Valkirs et al. 2003), and few regulations target the release and dumping of copper during paint application, cleaning and removal (but see ANZECC 1997, Kotrikla 2009). Restrictions on copper release rates have been introduced in Canada and Denmark. Health Canada requires that AF paints containing copper have a release rate of less than  $40\mu\text{g}/\text{cm}^2/\text{day}$  (HC 1994) and in Denmark AF paints that release copper exceeding a cumulative  $200\mu\text{g}/\text{cm}^2$  over the first 14 days are prohibited (DEPA 2003). Another strategy has been to target particular vessels or ecologically sensitive areas with restrictions e.g. copper AF paints are no longer in use on small boats on the Baltic Coast of Sweden (KEMI 2006). The US EPA is also reviewing current AF regulations with the likely scenario being restrictions on copper use in the future (Carson et al. 2009).

#### *'Booster' biocides*

Despite copper's high toxicity to many marine organisms, some algal groups are tolerant (Foster 1977, Reed and Moffat 1983). Hence, most copper AF paints are fortified with additional 'booster' biocides to target hull colonisation by micro- and macro-algae (Readman et al. 1993, Voulvoulis et al. 1999). Approximately eighteen compounds are used as biocidal additives worldwide (Thomas 2009). These are often herbicides (e.g. Irgarol 1051® and diuron) that have negative effects on the growth rate of photosynthetic organisms (Voulvoulis et al. 1999). Legislation now exists in some countries to regulate the use of some 'booster' biocides in AF paints, e.g. diuron and Irgarol 1051. In the UK, a review of booster biocides in 2000 resulted in only four

biocides gaining approval (dichlofluanid, DCOIT (Trade name: Sea Nine 211), zinc pyrrithione and zineb). Approvals of chlorothalonil, diuron and Irgarol 1051 were revoked due to their high toxicity at low concentrations and their persistence in the environment (Cresswell et al. 2006, Thomas 2009). Irgarol 1051 and diuron are also banned in Denmark (DEPA 2008), and diuron is banned in the Netherlands (Bannink 2004). Use of Irgarol 1051 in antifouling paints is not permitted in Australia as it was not granted approval for use as an antifouling biocide by the Australian Pesticides and Veterinary Medicines Authority (APVMA) when assessed in the 1990s. Under the European Union Biocidal Products Directive 2000 (BPD) all antifouling biocides are approved after demonstration that, when used as intended, the biocide presents no unacceptable risk to humans or the environment (Pereira and Ankjaergaard 2009). Applications for approval have been submitted for eleven antifouling biocides, including copper (II) oxide, copper thiocyanate and Irgarol, but not diuron (Pereira and Ankjaergaard 2009).

Increased awareness of the impacts resulting from the use of toxic AF paints has prompted investment in the research and development of non-toxic alternatives such as foul-release coatings that incorporate silicone elastomers, waxes or silicone oils, and “natural” coatings that source AF compounds from algae and other marine organisms (Clare 1998, Hellio et al. 2009). Foul-release coatings currently on the market include silicone (e.g. Intersleek 700<sup>®</sup>, Sealion<sup>®</sup> and Bioclean<sup>®</sup>), fluoropolymer (e.g. Intersleek 900<sup>®</sup>), hybrid (e.g. Phasecoat UFR<sup>®</sup>) and hydrogel silicone (e.g. Hempasil X3<sup>®</sup>) coatings (Townsin and Anderson 2009). “Natural” coatings however are not currently in commercial use due to the difficulties in sourcing a supply of natural AF compounds

at a reasonable cost in addition to meeting the requirement of environmental regulation agencies (reviewed by Yebra et al. 2004).

### **Environmental impacts of antifouling paints**

By necessity antifouling biocides are inherently toxic, and the widespread use of biocides in AF paints has introduced high levels of contamination into the environment and raised concerns about their toxic effects on marine communities. Toxicity is related to the properties of the contaminant as well as their bioavailability in the marine environment. For example, organotins such as TBT are highly toxic because of their increased fat solubility (compared to inorganic tin), which allows them to penetrate biological membranes (Champ and Pugh 1987). Toxicity will also increase if the contaminant is more bioavailable and this is related to local environmental conditions (e.g. temperature and pH) as well as the partitioning behaviour or binding strength of the contaminant to sediment (Eggleton and Thomas 2004). For example, copper bioavailability can increase with increasing temperature (Sokolova and Lannig 2008, Richards and Chaloupka 2009) and is reduced if the copper is strongly bound to organic compounds and sediments (Madsen et al. 1999). Therefore when contaminants occur in the water column, they are often found in their most toxic dissolved form whilst sediments tend to act as a contaminant sink (Flemming and Trevors 1989, Eggleton and Thomas 2004). The remobilisation of sediments by natural (e.g. storms) or anthropogenic events (e.g. dredging) can be a major source of contaminants but has received comparatively little attention (but see Eggleton and Thomas 2004, Knott et al. 2009).

Contaminants can enter the marine food chain through the lower trophic levels. Micro- and macro-algae absorb contaminants through diffusion into their tissues and invertebrates take up contaminants through permeable body surfaces and by ingestion of contaminated material e.g. filtering sediment from the water column or bioturbating benthic sediments. The transfer of contaminants to higher trophic levels is also of major concern, especially when the organism in question is an important human food source (Keithly et al. 1999). If the uptake of the contaminant exceeds the organism's ability for excretion and detoxification, this can reduce normal metabolic functioning (Rainbow 2007). Most organisms have physiological responses to cope with contaminant exposure, but these can come at a significant cost as energy is diverted away from essential metabolic functions towards detoxification (Sokolova and Lannig 2008). However, in cases where an organism is particularly tolerant, or possesses an opportunistic life strategy, there is the potential for them to dominate a community recovering from contamination or disturbance (Hall and Frid 1995, Johnston et al. 2002).

#### *Tributyltin*

Ecological effects on growth, development, reproduction and survival of TBT have been reported from a wide range of organisms ranging from bacteria to fish and mammals (Hoch 2001). Research has tended to focus on molluscs (gastropods and bivalves) because of their economic importance and as a group known to be heavily impacted by the use of TBT (Alzieu 1991, 1998). Laboratory studies indicate TBT exposure as low as 0.002 µg/L causes shell abnormalities in oysters by inhibiting calcification (Chagot et al. 1990), and > 0.73 µg/L results in reduced oyster growth (Valkirs et al. 1987). TBT monitoring in the water column along the French coastline



found concentrations were as high as 1.5 µg/L, which could have resulted in oyster shell and growth abnormalities (Alzieu et al. 1989). These findings generated significant concern and placed TBT in the world spotlight (Champ and Pugh 1987).

During this period, it became apparent that some gastropod populations were also declining in areas of high vessel activity and TBT was identified as the primary cause behind their disappearance (Bryan et al. 1986). Tributyltin causes a hormonal imbalance in gastropod females which induces the development of male sex organs, a condition known as imposex (Smith 1981, Matthiessen and Gibbs 1998). It should be noted that recent research has suggested that imposex may also be a response to other stress stimuli such as polychlorinated biphenyls (Garaventa et al. 2006, Garaventa et al. 2008), but TBT is still widely recognised as the primary cause. Imposex manifests in stages, initially with the development of a rudimentary penis and vas deferens in the female. This is followed by penis enlargement and the completion of the vas deferens which blocks the oviduct, preventing the release of eggs and potentially causing death (Gibbs and Bryan 1986). Tributyltin levels in the water column as low as 0.02 µg/L will readily induce imposex (Gibbs and Bryan 1986) and between 1.2 – 5.6 µg/L can induce irregular swimming behaviour of gastropod larvae (Horiguchi et al. 1998). TBT was first used as a freshwater molluscicide, therefore its damaging effects on bivalves and gastropods are not surprising. However the magnitude and extent of effect appears to have been substantially greater in the marine environment. One of the contributing factors has been the greater toxicity of TBT in saltwater compared to freshwater (Harino et al. 2000, Leung et al. 2007) and the persistence of TBT in the marine environment (Clark et al. 1988). These issues were not fully considered when TBT coatings were first developed and approved because TBT rapidly degrades in the marine environment,

with an estimated half-life of 6 – 9 days in seawater. However, the half-life of TBT varies considerably with changing environmental conditions and degradation slows considerably in oligotrophic environments (several years Michel and Averty 1999) and in sediments (16 wks to 3.5 yrs Ko et al. 1995, Lewis 1998).

Marinas and commercial ports in developed countries were identified as hotspots of TBT contamination with concentrations in the surface water and sediments correlating to the level of shipping or boating intensity (Valkirs et al. 1986, Cleary and Stebbing 1987, Batley et al. 1989). Recreational marinas have recorded TBT concentrations up to 0.93 µg/L in San Diego Bay (Valkirs et al. 1986), up to 1.06 µg/L in SW England (Cleary and Stebbing 1987) and 1.05 µg/L in Hong Kong (Lau Wong 1991). Less heavily impacted areas included SE Australia with TBT concentrations in the water column up to 0.19 µg/L in Sydney Harbour (Batley et al. 1989). Following the implementation of TBT regulations for vessels < 25 m, contamination became largely restricted to commercial vessel areas (Wilson et al. 1993, de Mora et al. 1995, Shim et al. 2000, Gibson and Wilson 2003, Andersen 2004, Harino et al. 2007). In coastal areas of Korea TBT contamination is greatest in areas of intense commercial shipping, up to 0.16 µg/L (Choi et al. 2009), and in Japan up to 0.04 µg/L in the Port of Osaka (Harino et al. 1998). Some developing countries where TBT remains unregulated have also experienced high levels of contamination (Rilov et al. 2000, Barakat et al. 2001, Bhosle et al. 2004, Sudaryanto et al. 2004).

Studies on the effects of tributyltin have included a range of organisms (for a comprehensive review of TBT acute toxicity see Hall Jr and Bushong 1996) and highlighted the magnitude of the global impact. The effects of TBT sediment

contamination have been found to extend into deep sediments and along major shipping lanes [0.019 µg/g (Strand et al. 2003)] and in 'pristine' marine environments, including Antarctica [2.2 µg/g (Negri et al. 2004)] and the Great Barrier Reef World Heritage Area [at a ship grounding site 340 µg/g (Haynes and Loong 2002)]. Long range passive transport of TBT in the environment has resulted in the exposure of organisms not inhabiting point source areas. For example, deep sea fish collected between 1000-1800 m depth in the Mediterranean had comparable levels of TBT to coastal fish (Borghi and Porte 2002). The effects of TBT contamination also have the potential to extend to higher organisms through consumption (Bhosle et al. 2004). Both mammals and seabirds have been found to harbour high TBT concentrations [e.g. cetaceans in Norway (Berge et al. 2004), Poland (Kannan and Falandysz 1997), Japan (Iwata et al. 1995) and the Mediterranean (Kannan et al. 1996) and river otters in the USA (Kannan et al. 1999)]. Tributyltin contamination in human blood and liver has since raised concerns about the trophic transfer of TBT via human consumption of seafood (Antizar-Ladislao 2008). Tolerable daily intake values for TBT of 0.25 µg/kg of body weight per day are recommended (Penninks 1993) and there is evidence that these could be exceeded from some seafood in fish markets (Belfroid et al. 2000). Consumption rates are not likely to be high enough to be damaging in most people (Keithly et al. 1999), but for some specific groups (e.g. fishers), in certain localities, TBT contaminated seafood may pose a health risk (Chien et al. 2002).

Since the introduction of TBT restrictions, there is some evidence of recovery in marine ecosystems. For example, the incidence of imposex has declined in the dogwhelk *Nucella lapillus* (L.) in the UK (Evans et al. 1991, Morton 2009) and Canada (Tester and Ellis 1995) and in the dog winkle *Dicathais orbita* (Gmelin) in Australia (Rees et

al. 2001). Oyster populations have recovered from TBT contamination in Australia (Batley et al. 1992, Batley 1995) and in France (Ruiz et al. 1996). However, in some areas imposex persists (e.g. Gibbs 2009).

A recent study by Dafforn et al. (2009a) found persistent TBT contamination in the water column in large recreational marinas (with levels that could potentially result in shell deformities in oysters). This is an interesting result considering TBT has been banned on recreational vessels for the last two decades and raises questions concerning the source of this contamination. Even a brief exposure to TBT can result in shell deformities and significant bioaccumulation in oysters (Scammell et al. 1991), therefore if a few recreational boats are still illicitly coated with TBT this might explain the results. However, with application of TBT paints to small craft (<25 m) banned in most Australian States in the late 1980s, and sales and application to any vessel prohibited in Australia from early 2003, a more likely scenario relates to the environmental persistence of TBT. Tributyltin has a half-life of just a few days in the water column (Seligman et al. 1996), being quickly broken down by bacterial metabolism and microalgae (Seligman et al. 1986, Seligman et al. 1988). In sediments, TBT can persist for months to years (Harris et al. 1996), and potentially much longer if deposited within paint flakes. If there is no ongoing illegal use of TBT, then detectable levels of TBT in the water column around recreational marinas likely result from TBT desorption from bottom sediments either by resuspension e.g. during shipping movements or coastal developments such as dredging (Nayar et al. 2004, Tolhurst et al. 2007, Hedge et al. 2009, Knott et al. 2009).

Tributyltin persistence in marine sediments has the potential to result in prolonged environmental damage (Clark et al. 1988, Hoch 2001), however the long-term effects of contaminated sediments on marine systems remains relatively unknown. Recreational marinas are of particular concern as they are often sheltered and poorly flushed (Floerl and Inglis 2003) making it less likely that contaminants accumulating in the sediment will be dispersed. This has implications for infauna that ingest and burrow into the contaminated sediments (Bartlett et al. 2004). Field data suggests that TBT sediment concentrations between 0.1 – 1 µg/g will have adverse effects on infaunal species and there is evidence of much higher TBT concentrations in the environment (Bryan et al. 1986, Langston and Burt 1991). Sediments collected from estuarine samples in Spain revealed high TBT concentrations ranging up to 5.48 µg/g in areas with historical industrial and fishing activities (Arambarri et al. 2003). Surface sediment TBT concentrations were also high at sites of intense shipping activity in India, up to 16.82 µg/g (Bhosle et al. 2006). While TBT remains bound to the sediments, impacts are likely to remain confined to the resident infaunal community, but contaminated sediments can also become a problem for epifaunal species if disturbance results in remobilisation of the contaminant (Svavarsson et al. 2001).

#### *Copper*

Copper is found naturally in the marine environment and is essential for healthy metabolic functioning as well as the growth and metamorphosis of many organisms (Bryan 1971, Lewis and Cave 1982, Hall Jr et al. 1998). Copper only becomes toxic when, in a bioavailable form, it exceeds the threshold of the organism's tolerance, and this has been shown to vary widely between species even within the same functional group (Piola and Johnston 2006, Han et al. 2008). Several studies have also suggested

that sensitivity to copper is greater during the early life stages of marine organisms (Xie et al. 2005).

Natural background concentrations of copper are estimated at between 0.5 – 3 µg/L, but in highly contaminated areas such as marinas, concentrations of up to 21 µg/L Cu have been found (Schiff et al. 2004). The bioavailability of copper in renders estimates from seawater largely unrepresentative of the potential toxicity (Thomas and Brooks 2010), therefore bioindicator species are often used to assess contamination levels.

Environmental monitoring in France found an increase in the copper content of oysters up to 87 mg/kg wet wt (after initial TBT restrictions on small vessels) near marinas and moorings (Claisse and Alzieu 1993). There is also evidence that copper emissions from recreational vessels are becoming a problem in more ecologically pristine areas with models predicting increases in toxicity levels up to 0.04 µg/L each year in the Great Barrier Reef World Heritage Area (Saphier and Hoffmann 2005).

To identify ecologically significant levels of copper and advise water quality guidelines, a number of lethal and sublethal effects of copper have been documented. Early observational studies identified an association between ‘green’ oysters and copper exposure (Boyce and Herdman 1897), although the potential negative effects of this have yet to be confirmed. More often, experimental studies are used to assess the toxic effect of copper on a particular species e.g. 1.2 µg/L Cu can reduce the filtration rate of a marine bivalve (reviewed by Hall Jr et al. 1998) and 20 µg/L Cu can impair or inhibit the settlement of coral larvae (Reichelt-Brushett and Harrison 2000). Copper toxicity is also related to reductions in photosynthetic activity and growth in marine algae (Nielsen and Wium-Andersen 1970, Overnell 1976, Wong and Chang 1991, Cid et al. 1995).

Diatoms in particular exhibit reduced growth (50 % at 100 µg/L Cu) (Cid et al. 1995) and oxidative stress, which can result in cell abnormalities (Rijstenbil et al. 1994). These studies all document the potential effects of copper accumulation in the water column, but depending on environmental condition, copper can be rapidly bound to sediments where its toxic effects can impact on infaunal communities (Chen et al. 2002).

Copper in sediments has been found to reduce the abundance of most taxa, but the size of response varies for different groups [e.g. crustaceans more sensitive than polychaetes (Stark 1998)]. Field monitoring at Casey Station in Antarctica found sediments contaminated with 30 µg/g Cu were associated with reduced diversity of infaunal species (Stark et al. 2003), and experimental spiking of sediments above background levels in temperate regions also resulted in reduced abundances of infaunal species (Hall and Frid 1995, Morrissey et al. 1996). Similarly, Rygg (1985) observed that Norwegian communities were less diverse where copper exceeded 200 µg/g. These results provide evidence that copper plays a major role in structuring infaunal communities by reducing recruitment of benthic species. Olsgard (1999) found that sediment spiked with 300 µg/g Cu reduced the recolonisation success of several polychaete species, a bivalve and brittle star in Norway. When these studies are considered in the context of potential estuarine contamination levels (between 1000 – 2400 µg/g Rygg 1985, Bryan and Langston 1992, Irvine and Birch 1998), the continued use of copper can be viewed as a major threat to soft sediment communities.

A recent risk assessment on the use of copper as a biocide in antifouling paints considered the concentration, speciation and effects of copper in the coastal marine

environment, and inputs from antifouling paints (Brooks and Waldock 2009). This concluded that copper toxicity was a potential problem only in isolated water bodies, such as enclosed marinas and harbours with little water exchange and high levels of boating activity. Despite assessing the overall environmental risk of copper from antifouling paints as low, Brooks and Waldock (2009) did recommend ongoing development of new improved and environmentally friendly antifouling products that would reduce copper usage and the risk to susceptible water bodies.

#### *'Booster' biocides*

Macroalgae have a relatively high tolerance to copper and 'booster' biocides initially were introduced to AF paints to improve their efficacy against these photosynthetic organisms (Voulvoulis et al. 1999). Many different 'booster' biocides have been added to AF paints including chlorothalonil, dichlofluanid, Irgarol 1051<sup>®</sup>, TCMS pyridine, thiocyanatomethylthio-benzothiazole (TCMTB), diuron, dichloro-octyl-isothiazolin (DCOIT, Sea Nine 211<sup>®</sup>), zinc and copper pyrithione (Zinc and Copper Omadine<sup>®</sup>) and zineb (Voulvoulis et al. 1999, Boxall et al. 2000, Thomas 2001, Thomas 2009). Four of the most widely used and studied 'booster' biocides are reviewed here.

Irgarol 1051 (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine) is an effective photoinhibitor and prevents algal fouling by decreasing electron transport within chloroplasts of photosystem II (Hall Jr and Gardinali 2004), but its effects have been found to extend to non-target species such as corals, mangroves and seagrasses, even at very low concentrations (Carbery et al. 2006). These effects are exacerbated by the continued persistence of Irgarol 1051 in the marine environment [half-life of around 100 days in seawater (Konstantinou and Albanis 2004)], and because several



degradation products (e.g. M1, M3) demonstrate even greater toxicity than the parent compound and longer persistence in the environment (Okamura et al. 2000).

Irgarol 1051 has been detected in estuaries worldwide and hotspots of contamination are linked to vessel activity (Readman et al. 1993, Dahl and Blanck 1996, Sargent et al. 2000, Konstantinou and Albanis 2004, Carbery et al. 2006). A recent study by van Wezel and van Vlaardingen (2004) suggests that Irgarol 1051 poses an environmental risk at levels above 0.024 µg/L and this value has already been exceeded in many areas worldwide, e.g. the Caribbean [up to 1.3 µg/L (Carbery et al. 2006)], Sweden [up to 0.4 µg/L (Dahl and Blanck 1996)], the United Kingdom [0.13 µg/L in poorly flushed marinas (Scarlett et al. 1997)], France [up to 1.7 µg/L near yachting marinas (Readman et al. 1993)] and the United States [up to 0.3 µg/L in marinas around San Diego (Sapozhnikova et al. 2007)]. Sediment resuspension has recently been highlighted as a source of ongoing Irgarol 1051 contamination; resulting in concentrations up to 0.23 µg/L with negative effects on the photosynthetic activity of the green alga *Ulva intestinalis* (Tolhurst et al. 2007). Several countries have imposed restrictions on the use of Irgarol 1051 (e.g. Australia, UK and Denmark) because of its perceived threat to primary producers, however the biocide continues to be widely used elsewhere.

Comparatively less is known about the other main ‘booster’ biocides; diuron, copper/zinc pyrithione or DCOIT. Diuron also inhibits photosynthesis by blocking the electron transfer in photosystem II (Giacomazzi and Cochet 2004). The biocide remains viable in the environment for a month to a year (Giacomazzi and Cochet 2004) and is more toxic to organisms such as the seagrass *Zostera marina* than Irgarol 1051 (Chesworth et al. 2004). Diuron has been shown to impact on the growth of

seagrasses and concerns have been raised on the environmental impacts of inshore contaminant levels of diuron attributed to agricultural run-off rather than antifouling usage (Haynes et al. 2000).

Pyrrithiones have a broad spectrum of antimicrobial activity and have been widely used as bactericides, fungicides and algaecides due to their low water solubility and favourable environmental chemistry (Turley et al. 2000). The compounds are active against fungal cell walls, associated membranes and bacterial (e.g. *Escherichia coli* and *Pseudomonas aeruginosa*) transport processes (Dinning 1998). They also rapidly degrade in the water column by photolysis, biolysis and sediment catalysed oxidation and reduction to less toxic compounds, with a reported half-life of <24 h (Turley et al. 2000). DCOIT is currently considered to have low environmental risk among ‘booster’ biocides because of rapid degradation (Thomas 2009). The compound has potential effects against a wide range of bacteria, fungi and algae and also rapidly degrades to compounds of negligible toxicity when released to the environment [estimated half-life of less than 24 h (Jacobson and Willingham 2000, Larsen et al. 2003)]. In the UK the use of DCOIT is restricted to professional use due to concerns to human health during application (HSE 2004, Thomas 2009).

‘Boosters’ have sometimes been assumed as environmentally sound, but their effects are poorly understood. We know that accumulations of booster biocides can reduce the germination and growth of non-target algae such as *Hormosira banksii* (e.g. diuron, zineb, DCOIT and zinc pyrithione Myers et al. 2006) and seagrasses such as *Zostera marina* (e.g. Irgarol 1051 and diuron Chesworth et al. 2004), reduce the photosynthetic efficiency of symbiotic algae in corals (e.g. Irgarol 1051 Carbery et al. 2006) and are

toxic to sea urchin eggs and embryos (e.g. DCOIT, zinc and copper pyrithione Kobayashi and Okamura 2002). However, knowledge gaps still exist regarding the interactive effects of different biocides, their occurrence, degradation, bioaccumulation and transport leading many researchers to recommend the precautionary principle when dealing with the regulation of these chemicals (Thomas 2001).

### **Role of antifouling paints in the transfer and establishment of non-indigenous species**

Heavy metals in the marine environment have long been recognised as important selection agents acting on aquatic organisms (reviewed by Klerks and Weis 1987). Estuaries tend to be highly contaminated with heavy metals and, along with industrial discharges, the AF paints applied to vessel hulls are an important source of these metals (Hall Jr and Anderson 1999). Many of the organisms living in these areas are non-indigenous and have arrived in hull fouling communities (Carlton 1987, 1989, Carlton and Geller 1993, Gollasch 2002, Godwin 2003, Minchin and Gollasch 2003). Recent studies suggest that the use of metals in AF paints and their accumulation in estuaries may in fact have facilitated the invasion of some metal tolerant NIS, by enabling their settlement and survival on vessel hulls painted with AF biocides, and also their establishment in polluted harbours (Piola and Johnston 2008a).

#### *Tributyltin*

Tributyltin is widely recognised to have been the most effective AF biocide and therefore it has been argued that the use of TBT rendered the threat of invasion from hull fouling organisms largely non-existent (Minchin and Gollasch 2003). Microalgae and bacteria were the only organisms to demonstrate a high degree of TBT tolerance.

Bacteria from the genera *Vibrio* and *Alteromonas* develop TBT tolerance after extended exposure (Suzuki et al. 1992, Suzuki and Fukagawa 1995) and *Bacillus* sp. collected from Boston Harbour sediments were more tolerant of TBT than bacteria collected from an uncontaminated source (Wuertz et al. 1991). Other tolerant biofouling organisms include diatoms from the genera *Achnanthes*, *Amphora* and *Amphiprora*, which have been found to colonise TBT AF paints (Callow 1986, Thomas and Robinson 1987, Cassé and Swain 2006).

The possible contribution that TBT AF paints to the translocation of non-indigenous species was a consequence of their longer life and the increased time between ship dry-dockings. Despite the presence of effective AF systems on ships' outer hulls, fouling species are known to be able to colonise "niche areas", which are parts of the vessel that are either not painted, are prone to paint damage, or are protected refuges where the AF does not work effectively. Sea chests, for example, can accumulate diverse, well-developed fouling communities (Coutts and Dodgshun 2007). The longer docking cycles afforded by TBT AF paints were conducive to establishment and reproductive maturation of such communities.

For organisms transported on a vessel hull to establish in a new region they must survive the local environmental conditions (Ruiz et al. 1997). Minchin and Gollasch (2003) suggest that the accumulation of TBT in estuaries has largely prevented the establishment of NIS due to its high toxicity, and future reductions in TBT levels due to restrictions on its use would result in greater opportunities for invaders. There is some evidence to support this claim, e.g. the barnacle *Amphibalanus variegatus* was introduced to Japan in 1936, but after 1970 the barnacle was not found again until a

2004 survey (Horikoshi and Okamoto 2005). This timeline of occurrence closely parallels the introduction of TBT in the 1960s and its gradual phasing out. One possible scenario is that elevated levels of TBT in Japanese harbours at the height of TBT use resulted in its disappearance (Dafforn et al. 2009a) and recent reductions in TBT contamination as a result of the ban have enabled its reappearance. Recent incursions of the serpulid tubeworm *Hydroides sanctaecrucis* and the Asian green mussel *Perna viridis* into northern Australia may also reflect this scenario (Lewis et al. 2006, Stafford et al. 2007). When coupled with the increase in copper accumulation in estuaries as a result of the TBT ban, we are likely to see an increasing shift towards copper-tolerant NIS dominance in estuarine biofouling communities (Piola and Johnston 2008a).

#### *Copper*

Copper AF paints are highly effective against most species after application, however when release rates drop below a critical level, it becomes likely that copper-tolerant species will recruit. This critical level appears in general to be higher for marine invaders than for endemic species (Allen 1953, Wisely 1963, Floerl et al. 2004, Dafforn et al. 2008, Piola and Johnston 2008a, Dafforn et al. 2009a). Areas where the AF coating is damaged on the hull, and uncoated surfaces, will also experience reduced exposure to leached copper and are likely to be colonized by copper-tolerant species (Piola and Johnston 2008b).

Copper tolerance can affect all stages of the biofouling ‘sequence’. Natural marine bacteria develop copper tolerance when exposed to moderate levels of the contaminant (Vaccaro et al. 1977), which makes them likely colonizers of copper AF paints. Many different algal species also develop resistant populations when exposed to copper

contamination including *Fucus vesiculosus* (Bryan and Gibbs 1983) and *Chlorella vulgaris* (Foster 1977, Butler et al. 1980). Han et al. (2008) suggest that copper tolerance may be an advantage for the alien *Ulva armoricana* over the native *Ulva pertusa* in the increasingly polluted estuaries of Korea. Copper tolerance is also associated with the transport of *Ulva (Enteromorpha) compressa*; ship fouling isolates were found to be tolerant of higher copper concentrations than non-fouling isolates (Reed and Moffat 1983). The brown alga *Ectocarpus siliculosus* is another prominent ship fouler and Russell and Morris (1972) suggest that copper tolerance evolves in populations whose habitat conditions are associated with high copper concentrations. This adds weight to the argument that copper tolerance facilitates the presence of species in polluted harbours and can also enable the transport of these species on hulls painted with copper AF paint.

Among the invertebrate foulers, attention has focused on several copper-tolerant species. As early as the 1950s, researchers recognized the potential for copper-tolerance to facilitate the transport of non-indigenous species on vessel hulls (Allen 1953). The bryozoan *Watersipora subtorquata* can attach to copper-painted vessel hulls and provide a toxic barrier, allowing less tolerant organisms to settle on the bryozoans surface (Fig. 3.2) (Allen 1953, Floerl et al. 2004). Larvae of the bryozoan *Bugula neritina* also attach directly to copper AF paints (Floerl et al. 2004), but their attachment strength is reduced by copper exposure (Wisely 1963). The cosmopolitan serpulid *Hydroides elegans* is also highly tolerant of copper (Allen 1953, Lewis and Smith 1991, Johnston and Keough 2003, Dafforn et al. 2008, Piola and Johnston 2008a, Dafforn et al. 2009a), and its spread around the world is thought to have occurred primarily through biofouling on vessel hulls (Pettengill et al. 2007). Similarly, *H. sanctaecrucis*

was first discovered in Australia on the hull of a vessel with depleted copper-based paints (Lewis et al. 2006). The serpulid *Salmacina australis* also responds positively to copper antifouling paints (Dafforn et al. 2008, Piola and Johnston 2008a, Dafforn et al. 2009a). Interestingly, *S. australis* is recognised as endemic to Australia, but its high tolerance to copper suggests its potential for export around the globe. Subsequent research has identified the potential for copper contamination to affect the establishment of marine organisms. Piola and Johnston (2008a) found that native species richness in four Australian locations was reduced by exposure to copper AF paints – whilst non-indigenous species richness was not affected. Hence the paints had the potential to switch a community from native dominated to invader dominated.

The translocation and introduction of NIS can feasibly be prevented at any stage of the translocation process, but the earlier the intervention the greater the effect. To this end, preventing the colonization of ships through close attention to the effectiveness, appropriateness and maintenance of antifouling systems is essential. Recent work suggests that we act at the start of the invasion pathway and increase the resistance of vessel hulls to colonization by non-indigenous species (Floerl and Inglis 2005, Lewis and Coutts 2009). To this end, combining information about donor regions with information about species metal tolerances could enhance predictions about potential exports.

#### *‘Booster’ biocides*

‘Booster’ biocides including Irgarol 1051, diuron, DCOIT and copper and zinc pyrithiones, were introduced to target algal slimes by inhibiting photosynthesis (Voulvoulis et al. 1999), but recent studies suggest the use and accumulation of these

biocides may also encourage the development of tolerance in marine communities.

Peterson et al. (2004) found that high concentrations of zinc pyrithione resulted in increased growth of tolerant microbial species, and exposure to diuron (Molander and Blanck 1992) or Irgarol 1051 (Blanck et al. 2009) can induce tolerance in marine diatoms. Similarly, exposure of microbial communities to DCOIT resulted in a loss of diversity with those species remaining able to tolerate the toxicant (Larsen et al. 2003). The continued use of 'booster' biocides in AF paints increases the likelihood that communities will develop increased tolerance and therefore reduce their effectiveness at preventing biofouling and the transport of non-indigenous species.

### **Future directions for antifouling paints and the management of their environmental impact**

Despite the recent regulation surrounding TBT, there are still problems of contamination around commercial ports and evidence of its continued presence in marinas (Gibson and Wilson 2003). Given the uncertainty surrounding TBT persistence in the environment (Seligman et al. 1996), remediation of highly contaminated sediments, particularly around large recreational marinas, seems a logical next step (Dafforn et al. 2009a). Despite its toxicity, many microorganisms (bacteria, algae and fungi) exhibit resistance to TBT and play an important role in the degradation of TBT to less toxic derivatives such as dibutyltin and monobutyltins, as well as the removal of TBT compounds by accumulation (Dowson et al. 1996, Gadd 2000). Deliberate inoculation of sediment with marine bacteria is a possible mechanism for TBT remediation as several species are able to degrade organotins (Gadd 2000). Microalgae could also play an important role in the biodegradation of TBT in the water column, but these organisms are limited in their activity by sunlight and nitrates (Lee et al. 1989).



While addition of nitrates to stimulate algal growth is a viable option (Lee et al. 1989), many of the more highly contaminated areas (e.g. marinas) are shaded by artificial structures, which could limit algal activity. Tributyltin remediation using microbial processes appears sound in theory, but is some way from commercial application due to the high toxicity of TBT in the field (Gadd 2000) and biological fixes are problematic with regards to bioinvasion (Myers et al. 2000). Moreover, this process could prove to be a lengthy one [in the order of several years (Dubey and Roy 2003)], and costly, which raises the issue of responsibility for remediating the TBT contaminated material (Champ 2003).

Copper AF paints are under scrutiny in many countries due to high levels of contamination in recreational marinas and the potential for future increases in commercial areas as a result of current AF regulations. Concern about the use of copper AF paints is related to the environmental impacts associated with metal build-up in estuaries as well as the risk of invasion from copper-tolerant fouling species (previously discussed). More recently, researchers have considered the effect of a changing climate on metal bioavailability, which raises concern about the future use of metals in AF paints. It is likely that the effect of metal contamination on marine organisms might be magnified by a changing climate, i.e. increased temperatures and ocean acidification. Temperatures are predicted to increase by 1.8 – 4°C by the year 2100 (IPCC 2007) and the increased sequestration of carbon dioxide by the ocean as a consequence of global warming is expected to lower pH levels in the order of 0.5 pH (RS 2005). Increased temperatures increase the solubility of metals and would likely result in faster rates of leaching from AF paints and a resultant reduction in durability (Dobretsov et al. 2005). Dissolved free metal ions are the most toxic form of metal and elevated temperatures

can therefore enhance the toxic effects of metals on marine organisms (Cairns et al. 1975). Increased ocean acidification may have indirect effects on marine organisms by modifying the chemistry of toxins such as trace metals and by increasing bioavailability (RS 2005).

Future increases in metal bioavailability through ocean acidification and global warming also have implications for the management of marine invasion. Increased dissolution of metals either through leaching from AF paints or release from sediments may have negative impacts on native marine communities and leave them susceptible to invasion by more metal-tolerant NIS. We agree that the preferable way forward is for the phase-out of metals and organic biocides from AF paints and to therefore promote research and development on non-toxic alternatives (Carson et al. 2009, Piola et al. 2009). However, we call for caution in the timeframe for implementing regulations, to prevent harmful ecological effects from banning metals before equally effective alternatives have been developed. While copper has been shown to enhance the invasion potential for some species, there is no evidence to suggest copper AF paints would promote the transport of more harmful invaders e.g. *Perna perna* or *Mytilopsis sallei* to Australia. Therefore unless other AF coatings prove to be highly effective, restricting the use of copper might increase the invasion opportunities for ‘high risk’ NIS on less effective AF coatings.

We propose following the strategy that resulted in the elimination of TBT from AF paints, beginning with the regulation of release rates and stricter regulation of dry dock facilities. Initial restrictions of TBT limited release rates to  $< 5 \mu\text{g}/\text{cm}^2/\text{day}$  and resulted in the removal of the more harmful conventional paints from the market, leaving only

SPC paints with more controlled release rates (Evans 1999). Conventional copper-based paints are still the most widely used by recreational vessel owners due to their affordability, but these also have greater polluting potential than SPCs (Valkirs et al. 2003). Therefore we suggest initially imposing limitations on copper release rates that would eliminate the conventional paints and continued monitoring to identify the effects of this form of regulation on copper loads in polluted hotspots.

The issues surrounding the use of biocides in AF paints have driven research in an environmentally friendly direction, with a particular focus on foul-release technology and natural marine compounds to inhibit biofouling. Foul-release coatings rely on silicone technology which does not prevent settlement, but reduces the strength of attachment of settling organisms. They are best suited to fast moving vessels as they generally require a speed of 20 knots or more to dislodge biofouling (Srinivasan and Swain 2007). Frequent cleaning would be necessary to prevent build up of biofouling on vessels that do not have the activity or speed to self-clean (Carson et al. 2009). Although promising, these coatings are currently expensive. The hull needs to be cleared of previous AF coatings and application of the foul-release coating is costly (Srinivasan and Swain 2007, Carson et al. 2009). However, these coatings do not leach and could prove to be more durable than copper-based paints for some vessel applications (Carson et al. 2009).

Although still in the early stages of development, paints containing naturally-produced compounds isolated from marine organisms also show promise as an environmentally friendly alternative to biocides (Hellio et al. 2009). Sources of natural AF compounds include the metabolites produced by sessile organisms such as cnidarians, ascidians,

sponges, algae and seagrasses produce. Bacteria isolated from organisms in the marine environment are also a possible source of natural AF compounds. For example, *Pseudoalteromonas tunicata* isolated from the tunic of the ascidian *Ciona intestinalis* produces 5 compounds that inhibit a range of organisms (Burgess et al. 2003), and bacterial strains isolated from the surface of the seaweed *Fucus serratus* and the nudibranch *Archidoris pseudoargus* were found to inhibit settlement by other micro-organisms (Armstrong et al. 2000). If these compounds can be incorporated into effective coatings, there is the potential to disrupt the early stages of biofilm development and therefore inhibit the settlement of some macrofauna (Burgess et al. 2003). Difficulties in this approach lie in the identification of compounds that inhibit a wide range of fouling organisms and the subsequent integration of these compounds into a coating that can be applied to a vessel hull (Yebra et al. 2004). “Natural” antifouling compounds also face the same regulatory hurdles to approval as any other new antifouling biocides with the estimated cost of assembling data packages on efficacy and environmental fate and effects many millions of dollars, and the timeline for the approval process of approximately 10 years (Rittschof 2000).

## Conclusion

Decisions regarding the use and regulation of AF paints in the marine environment are complex and require the integration of information regarding the economic and ecological costs and benefits of different strategies. Bans on TBT were primarily based on the economic costs of TBT to the oyster industry, but also because of ecological impacts to non-commercial species, bioaccumulation of TBT by a wide range of organisms, and potential human health risks. The subsequent shift to copper AF paints

containing 'booster' biocides may still result in environmental impacts, toxicity to non-target marine organisms and the spread of copper-tolerant NIS.

We have identified several knowledge gaps regarding the persistence of TBT in the marine environment and have highlighted the potential for the use of micro-organisms in remediation of TBT contaminated areas. Copper is present in the marine environment from many sources and so eliminating copper from AF paints is controversial (Johnson and Miller 2002, Srinivasan and Swain 2007, Carson et al. 2009, Piola et al. 2009). An ideal situation would allow the complete elimination of toxic biocides from AF paints, however, in reality this poses the question of what do we replace them with? We currently do not have a viable option for widespread replacement of copper in AF paints (although foul-release and natural antifouling compounds show some potential). Without an effective replacement, the ecological costs associated with invasion and economic costs associated with increased fuel consumption outweigh the impacts of current AF strategies. Therefore we would advise caution in regulating copper and the gradual introduction of increased restrictions, similar to the TBT strategy, to allow time for the development and implementation of more environmentally-friendly alternatives.



**Figure 3.1.** Vessel hull fouling. Marine biofouling describes the community of organisms that settle and grow on the external surfaces of submerged structures. Within hours of a structure's submergence, a slime layer develops. This layer promotes the settlement and attachment of macro-organisms, including larvae of invertebrates such as ascidians, serpulids and barnacles. Photo: J. Lewis.



**Figure 3.2.** Positive interactions between fouling species. The non-indigenous bryozoan *Watersipora subtorquata* is shown growing directly onto a vessel hull coated with copper AF paint and facilitating algal recruitment. Photo: J. Lewis.

## Chapter 4

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### Shallow floating structures promote marine invader dominance.

#### Abstract

Global increases in urban development have resulted in severe habitat modification in many estuaries. Most are now dominated by artificial structures, which might have a myriad of effects on native species. The provision of extra hard-substrate presents additional free space, and recent research suggests non-indigenous epifauna may be better able to exploit these artificial structures (particularly pontoons) than are native species. The early development of fouling assemblages was compared on settlement plates attached to fixed or moving experimental structures at depths of 0.5 m and 2 m. Invertebrate invaders as a group were disproportionately more numerous on shallow, moving plates (essentially floating surfaces) than on deeper plates, while native epifauna were less numerous than invaders in all treatments. Importantly, however, individual invasive species showed differing effects of movement and depth. Future management strategies should take into account the potential for shallow, moving structures to enhance invader dominance and strongly consider using fixed structures to reduce opportunities for invaders.

#### Introduction

The estuarine environment faces increasing pressure from encroaching urban developments. The addition of artificial structures such as seawalls, pilings and pontoons to urban waterways is of particular concern due to the potential effect of these

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#### *4. Shallow floating structures promote invader dominance*

structures on sessile epifauna. Comparisons of artificial structures and natural reefs in sheltered parts of Sydney Harbour have revealed distinct differences in the assemblages able to colonise and persist on these structures versus natural reefs (Connell and Glasby 1999, Bulleri and Chapman 2004). Intertidal assemblages on seawalls tend to be distinct from those on rocky reefs, supporting fewer species and being dominated by algae (Bulleri and Chapman 2004). Subtidally, assemblages on sandstone seawalls and reefs are also distinguished from pilings and pontoons by fewer taxa and a greater cover of coralline algae (Connell and Glasby 1999). Moreover, pilings and pontoons tend to support more bryozoans, ascidians and mussels than do rocky reefs (Glasby 1999a, Connell 2001). Differences between artificial structures and natural rocky reefs arise due to their physical characteristics, including substrate composition (Anderson and Underwood 1994, Glasby 2000, Chapman and Bulleri 2003), age (Perkol-Finkel et al. 2005), orientation (Connell 1999, Glasby and Connell 2001, Saunders and Connell 2001, Knott et al. 2004), predation levels (Nydham and Stachowicz 2007), illumination levels (Glasby 1999b) and due to the fixed nature of pilings, seawalls and reefs versus the movement of pontoons (Connell 2000, Holloway and Connell 2002, Neves et al. 2007, Perkol-Finkel et al. 2008, Shenkar et al. 2008).

Association with artificial structures is used as a criterion for identifying non-indigenous species (NIS) (Chapman and Carlton 1991), however the influence of these structures on invasions is still not fully understood. NIS can be a significant threat to ecosystems, as they have the potential to reduce native biodiversity through competition for resources, habitat alteration or predation (Mack et al. 2000, Holloway and Keough 2002). Ports and estuaries are major foci for arriving invaders and contain a large

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source population for further transport and dispersal (Carlton 1987, Floerl and Inglis 2005).

The addition of artificial structures to estuaries may assist invader establishment by creating space, a major limiting resource for hard-substrate assemblages (Sutherland 1974, Buss 1979, Russ 1982). Such structures also provide a greater diversity of habitat, both moving and fixed, which invading species may be better able to exploit than native species (Glasby et al. 2007). Bulleri and Aioldi (2005) found that invasion of seawalls by the alga *Codium fragile* ssp. *tomentosoides* was controlled by both disturbance (in the form of wave exposure) and biotic resistance from the superior native space occupier *Mytilus galloprovincialis*. Vaselli et al. (2008) highlight the potential for seawalls to alter water circulation patterns and aid the establishment of invasive species. Similarly, Glasby et al. (2007) found the greatest number of non-indigenous species on pontoons and the least on seawalls and rocky reefs where native species dominated space. Pilings and pontoons are of particular concern due to their increasing presence in boating estuaries (Glasby and Connell 1999), and their proximity to vessels. Vessel hulls are a major vector of introduction for sessile invertebrates (Gollasch 2002, Godwin 2003) and they present a similar habitat to pontoons as they float close to the water's surface at a constant depth. Neves et al. (2007) found that species richness and composition were similar between floating pontoons and vessel hulls within the same marina, suggesting the potential for transfer of species between these structures. In contrast, pilings are fixed structures that maintain contact with the seafloor, experience a range of depths and have been found to support different assemblages to pontoons (Connell and Glasby 1999).

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Past studies have investigated recruitment to experimental fixed and moving structures, but none has considered invader recruitment with respect to movement and depth, and variation in recruitment has rarely been examined over a small, shallow depth range. Perkol-Finkel et al. (2008) compared tropical assemblages between floating and fixed settlement plates and found compositional differences (at the level of functional group) relating to movement at depths of 1 – 12 m. Using a similar higher classification level, Holloway and Connell (2002) found swash and floatation contributed most to the development of a typical temperate pontoon fouling assemblage.

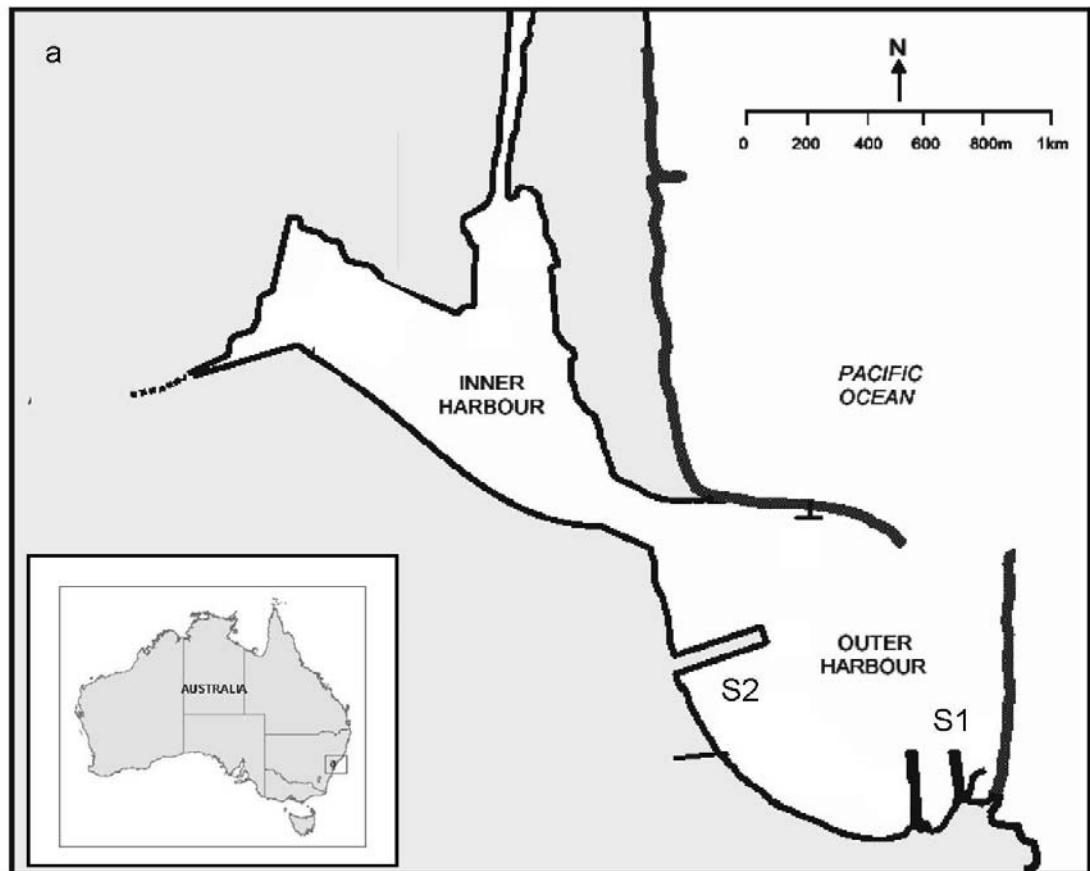
It has been hypothesised that non-indigenous species are most prevalent on floating structures like pontoons either because of proximity to the surface and/or because of movement (Glasby et al. 2007). This current study was designed to discriminate between these hypotheses with greater taxonomic resolution than used in previous studies, including classification of species as non-indigenous or native. The specific model tested was that the combined effects of movement and depth influence the richness of non-indigenous epifauna more so than native epifauna. NIS were predicted to be most numerous on shallow, moving substrates (analogous to a floating pontoon) and more numerous than native epifauna. The depths chosen for the study were 0.5 m and 2 m. The differential effects of moving and fixed structures on the recruitment and early development of fouling assemblages were tested while keeping constant other potentially confounding variables, for example age and type of substrate. The results are interpreted with both reference to species that are non-indigenous to Australia and species that are native to Australia, but have been introduced overseas; the latter are termed ‘exported’ species (ES). Hereafter, NIS and ES are referred to as ‘invaders’. For international relevance, it is important to consider invaders as a specific group

including not only those species that pose a threat to Australia, but also species originating from Australia that might pose a threat to other countries.

## **Methods**

### *Survey design*

Experiments were conducted in Port Kembla, New South Wales, Australia, between October 2006 and December 2006 (Fig. 4.1). Port Kembla is a major commercial harbour which receives approximately 600 local and international vessels/year (PKPC 2006). The harbour has a tidal range of 1.3 m and has a mixture of fixed and moving structures although the majority are fixed. To test for differences in the number of invaders on fixed or moving substrates, Perspex plates were attached to larger frames and deployed them vertically in the water column at two sites approximately 500 m apart. Moving frames had floats attached to allow them to remain at a constant depth below the surface despite changes in tides (similar to Holloway and Connell 2002, Fig. 4.2 a) and fixed frames were hung from jetties and weighted to remain stationary with the rise and fall of the tide (Fig. 4.2 b). All frames were deployed under the pier and experienced shaded conditions throughout the experiment. To test for differences in species richness and percent cover as a result of depth, moving frames floated at 0.5 m or 2.0 m below the water surface, whereas fixed frames were fixed at 0.5 m or 2.0 m below MLWS. Six (11 x 11 cm) replicate plates were deployed on two frames for each treatment combination (i.e. moving and shallow; moving and deep; fixed and shallow; fixed and deep. Plates were spaced ~ 20 cm apart on each frame.



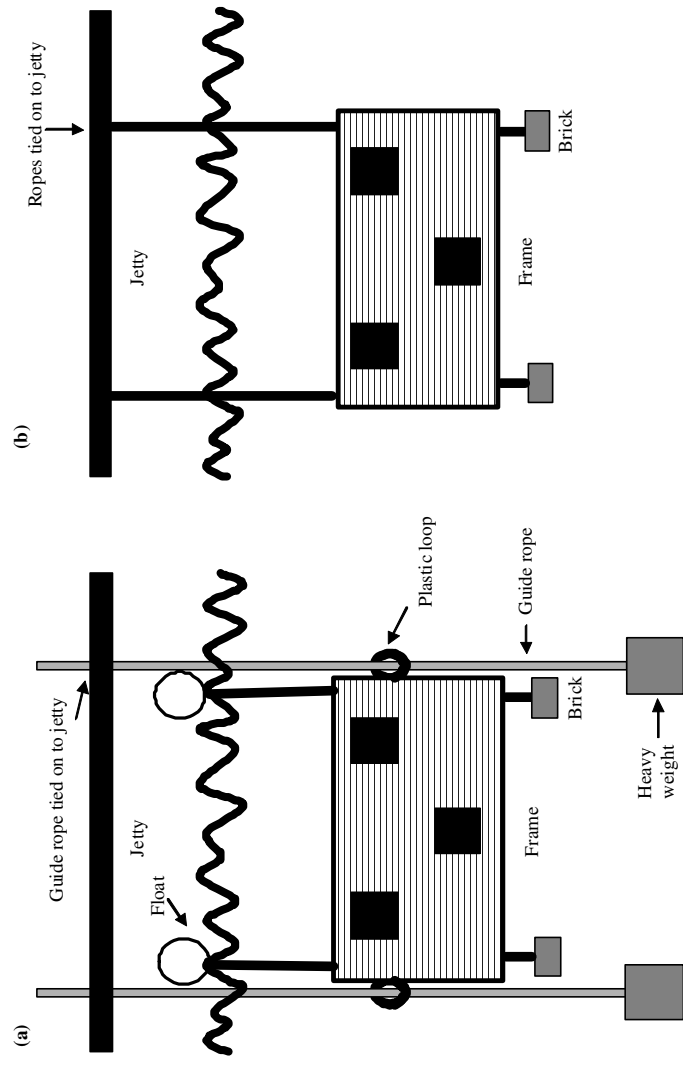
**Figure 4.1.** Locations of experimental sites in Port Kembla

#### *4. Shallow floating structures promote invader dominance*

To assess early fouling assemblage development, settlement plates were collected after 12 weeks when between 5-25% bare space remained. Plates were photographed and preserved in 5% formalin before counting. Percentage covers of each species were estimated using a grid of 81 regularly spaced points placed over the preserved plate with a 1 cm border to account for edge effects. Photographs were used to assist in the identification of organisms on the plates. Organisms in the grid, but not under a point were noted and given a value of 0.5 % cover. Organisms were identified to the lowest practical taxonomic level and classified as non-indigenous (NIS), exported (ES), native (N) or cryptogenic (C) [species without binomials (*sensu stricto* Carlton 1996a) or with binomials for which the origin is not presently resolved] according to the literature (Appendix II). Cryptogenic species were included in the total species pool for analyses. When considering invader richness, species non-indigenous to Australia and exported species that were invaders elsewhere were combined.

#### *Data Analyses*

The frequency of occurrence of invaders versus natives was compared for each treatment combination (i.e. moving and shallow; moving and deep; fixed and shallow; fixed and deep) using  $\chi^2$  tests. These tests related the frequency of occurrence to what would be expected by chance given the available species pool (number of NIS + ES + N + C). A three-factor ANOVA was used to compare percent covers of invader and native species between fixed and moving plates at different depths, and between sites. Movement and depth were treated as fixed orthogonal factors while site was considered random and orthogonal. All data were assessed for normality and homogeneity of variances using residual frequency histograms and Cochran's C test, respectively.  $\ln(x + 1)$  transformations were used to obtain homogeneous variances where necessary. SNK tests were used to identify significant differences between treatments. For native



**Figure 4.2.** Diagram of the apparatus used to create (a) moving and (b) fixed structures. Moving frames were deployed at either 0.5 m or 2 m below the water surface. Fixed frames were at either 0.5 m or 2 m below MLWS (and their depth therefore varied according to the tides).

species the main results are presented in-text and full ANOVA tables are included in Appendix V).

In several analyses, movement and depth effects were variable between sites. These small-scale differences have been interpreted in the text, but graphs have been presented for main effects only.

## Results

Thirty-nine taxa were identified during the study (most to genus or species). These included species of encrusting and arborescent bryozoans, barnacles, solitary and colonial ascidians, and serpulid polychaetes. Sixteen species were identified as non-indigenous to Australia, six as exported, nine as native and eight as cryptogenic (see Appendix 1 for full details). NIS alone were significantly more abundant than native species on all plates (Fig. 4.3 a; ANOVA:  $p < 0.01$ ). The frequency of occurrence of invaders and natives on shallow moving plates were significantly different from what would be expected by chance given the species pool (Fig. 4.3 a;  $\chi^2 = 5.23$ ,  $p < 0.05$ ). That is invaders occurred more frequently on shallow moving plates than would be expected by chance, and vice-versa for natives. No significant pattern was detected on deep moving plates ( $\chi^2 = 3.11$ ,  $p > 0.05$ ), shallow fixed plates ( $\chi^2 = 2.36$ ,  $p > 0.05$ ) or deep fixed plates ( $\chi^2 = 2.52$ ,  $p > 0.05$ ).

### *Effects of movement on invader recruitment*

Several species showed a positive response to movement. The non-indigenous ascidian *Diplosoma listerianum* (Milne-Edwards 1841) and exported ascidian *Herdmania momus* (Savigny 1861) had significantly greater percent covers on moving compared to



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fixed plates (Fig. 4.3 b, c; Table 4.1). The non-indigenous ascidian *Botrylloides leachi* (Savigny 1816), non-indigenous barnacle *Megabalanus coccopoma* (Darwin 1854) and exported barnacle *Amphibalanus variegatus* (Darwin 1854) also occupied more space on moving plates (Fig. 4.3 d, e, f; Table 4.1), but this was only significant at one site. In contrast, the exported barnacle *Balanus trigonus* (Darwin 1854) and the non-indigenous bryozoan *Microporella umbracula* (Audoin 1826) occupied significantly more space on fixed plates (Fig. 4.3 g, h; Table 4.1). Covers of the non-indigenous ascidian *Styela plicata* (Lesueur 1823) and bryozoan *Conopeum seurati* (Canu 1928) were also greater on fixed plates (Fig. 4.3 i, j; Table 4.1), but this was only significant at one site.

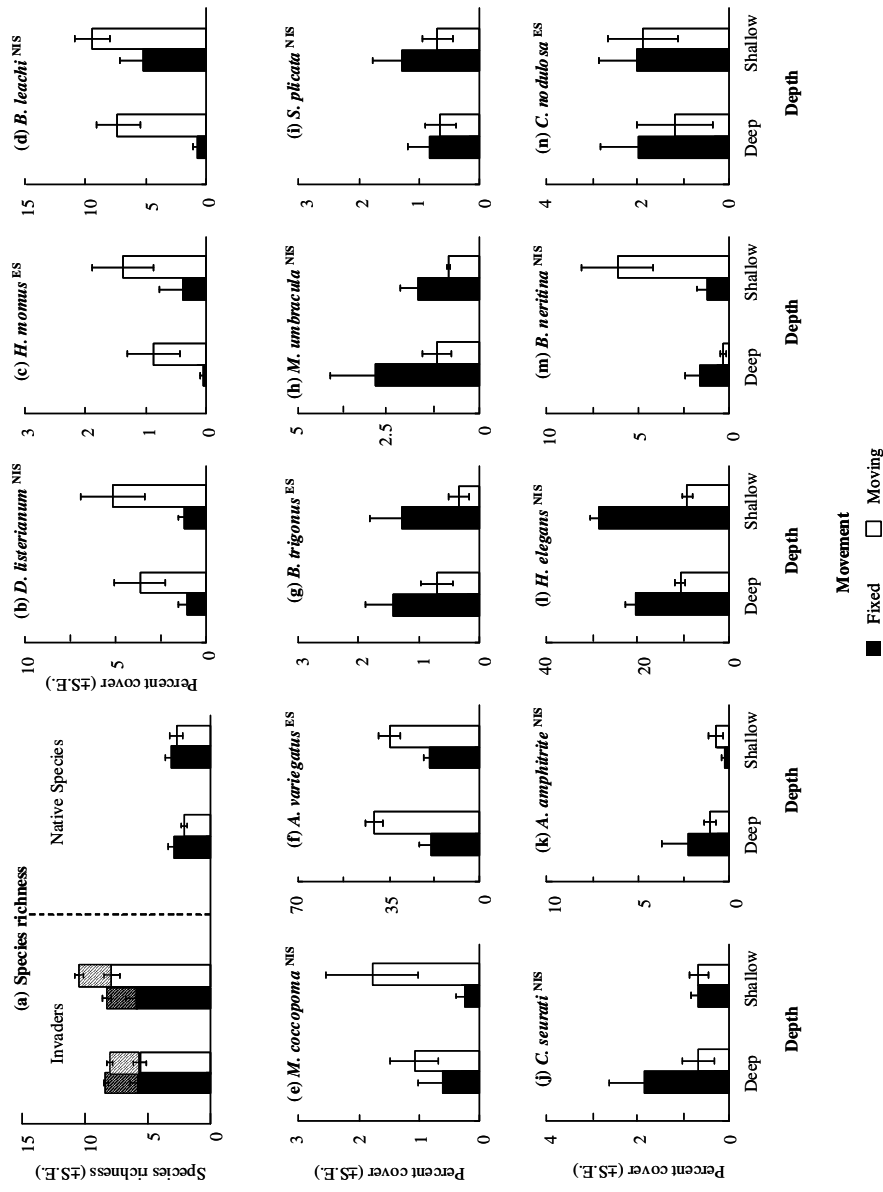
#### *Effect of depth on invader recruitment*

The non-indigenous barnacle *Amphibalanus amphitrite* (Darwin 1854) was the only species to show an overall effect of depth and occupied more space on deep than shallow plates (Fig. 4.3 l; Table 4.1). Percent covers of *H. momus*, *B. leachi* and *S. plicata* also varied with depth and were greater on shallow than deep frames (Fig. 4.3 e, f, i; Table 4.1), but this was only significant at one site. In contrast, *C. seurati* occupied more space on deep than shallow frames (Fig. 4.3 j; Table 1) although this again was only significant at one site.

#### *Interactive effect of movement and depth on invader recruitment*

Cover of the non-indigenous serpulid *Hydroides elegans* (Haswell 1884) varied according to movement, depth and site (Fig. 4.3 l; Table 4.1). The serpulid occupied almost twice as much space on fixed plates compared to moving plates regardless of depth and, at one site, had a greater cover on shallow fixed plates than on deep fixed plates. Cover of the non-indigenous bryozoan *Bugula neritina* (Linnaeus 1758) also

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**Figure 4.3.** (a) Species richness of invaders (NIS ¼ filled; ES ¼ hatched) and native species; (b)–(n) percentage cover of nonindigenous species (NIS) and exported species (ES) on fouling plates attached to fixed or moving frames and deployed at 0.5 or 2 m depth. Data were pooled for sites.

**Table 4.1.** Analyses of variance comparing percent covers of non-indigenous (NIS) and exported species (ES) on fouling plates that were attached to fixed or moving frames at different depths.

Source	df	MS	F	P	MS	F	P	MS	F	P	MS	F	P
<b>(a) <i>D. listerianum</i><sup>NIS</sup></b>													
				$\ln(x+1)$									
Movement	1	5.997	11.01	<b>0.002</b>									
Depth	1	0.433	0.79	0.378									
Site	1	1.089	2.00	0.165									
Mo x De	1	0.002	0.00	0.957									
Mo x Si	1	0.226	0.42	0.523									
De x Si	1	0.220	0.40	0.529									
Mo x De x Si	1	0.068	0.12	0.726									
Error	40	0.573											
<b>(b) <i>H. momus</i><sup>ES</sup></b>													
				$\ln(x+1)$									
Movement	1	2.099	9.02	<b>0.005</b>									
Depth	1	0.354	0.21	0.729									
Site	1	0.665	2.86	0.099									
Mo x De	1	0.013	0.02	0.913									
Mo x Si	1	0.007	0.03	0.867									
De x Si	1	1.718	7.38	<b>0.010</b>									
Mo x De x Si	1	0.688	2.96	0.093									
Error	40	0.238											
<b>(c) <i>B. leachi</i><sup>NIS</sup></b>													
				$\ln(x+1)$									
Movement	1	341.646	2.26	0.374									
Depth	1	129.338	0.90	0.517									
Site	1	1.864	0.10	0.753									
Mo x De	1	16.229	0.87	0.356									
Mo x Si	1	151.072	8.12	<b>0.007</b>									
De x Si	1	144.076	7.74	<b>0.008</b>									
Mo x De x Si	1	0.556	0.03	0.864									
Error	40	19.055											
<b>(d) <i>M. coco-poma</i><sup>NIS</sup></b>													
				$\ln(x+1)$									
Movement	1	2.107	1.63	0.423									
Depth	1	0.013	0.05	0.832									
Site	1	0.130	0.46	0.499									
Mo x De	1	0.357	1.28	0.265									
Mo x Si	1	1.292	4.62	<b>0.037</b>									
De x Si	1	0.326	1.17	0.286									
Mo x De x Si	1	0.034	0.12	0.730									
Error	40	0.285											
<b>(e) <i>A. variegatus</i><sup>ES</sup></b>													
				$\ln(x+1)$									
Movement	1	4143.055	2.82	0.342									
Depth	1	84.544	0.39	0.646									
Site	1	80.742	0.63	0.432									
Mo x De	1	131.177	1.02	0.318									
Mo x Si	1	1468.384	11.44	<b>0.002</b>									
De x Si	1	218.072	1.70	0.200									
Mo x De x Si	1	5.370	0.04	0.839									
Error	40	131.390											
<b>(f) <i>B. trigonus</i><sup>ES</sup></b>													
				$\ln(x+1)$									
Movement	1	1.522	5.90	<b>0.019</b>									
Depth	1	0.281	1.09	0.303									
Site	1	0.237	0.92	0.343									
Mo x De	1	0.018	0.07	0.791									
Mo x Si	1	0.121	0.47	0.497									
De x Si	1	0.002	0.01	0.924									
Mo x De x Si	1	0.003	0.01	0.912									
Error	40	0.274											
<b>(g) <i>M. umbracula</i><sup>NIS</sup></b>													
				$\ln(x+1)$									
Movement	1	2.268	4.70	<b>0.036</b>									
Depth	1	0.181	0.38	0.543									
Site	1	0.028	0.06	0.810									
Mo x De	1	0.008	0.01	0.949									
Mo x Si	1	0.125	0.26	0.614									
De x Si	1	0.300	0.62	0.435									
Mo x De x Si	1	1.263	2.62	0.113									
Error	40	0.496											
<b>(h) <i>S. plicata</i><sup>NIS</sup></b>													
				$\ln(x+1)$									
Movement	1	0.207	0.19	0.741									
Depth	1	0.164	0.16	0.756									
Site	1	2.544	12.17	<b>0.001</b>									
Mo x De	1	0.070	0.21	0.729									
Mo x Si	1	1.119	5.35	<b>0.026</b>									
De x Si	1	1.003	4.80	<b>0.034</b>									
Mo x De x Si	1	0.341	1.63	0.209									
Error	40	0.209											
<b>(i) <i>C. seurati</i><sup>NIS</sup></b>													
				$\ln(x+1)$									
Movement	1	0.664	0.53	0.600									
Depth	1	0.196	0.10	0.809									
Site	1	1.335	8.00	<b>0.007</b>									
Mo x De	1	0.445	2.66	0.110									
Mo x Si	1	1.258	7.53	<b>0.009</b>									
De x Si	1	2.043	12.24	<b>0.001</b>									
Mo x De x Si	1	0.058	0.34	0.561									
Error	40	0.170											
<b>(j) <i>A. amphirrite</i><sup>NIS</sup></b>													
				$\ln(x+1)$									
Movement	1	0.070	0.19	0.666									
Depth	1	1.808	4.86	<b>0.033</b>									
Site	1	0.115	0.31	0.581									
Mo x De	1	0.270	0.73	0.399									
Mo x Si	1	0.020	0.05	0.819									
De x Si	1	0.011	0.03	0.867									
Mo x De x Si	1	0.361	0.97	0.330									
Error	40	0.390											
<b>(k) <i>H. elegans</i><sup>NS</sup></b>													
				$\ln(x+1)$									
Movement	1	2533.493	107.93	<b>0.000</b>									
Depth	1	114.474	1.07	0.490									
Site	1	71.442	3.04	0.089									
Mo x De	1	250.354	1.33	0.455									
Mo x Si	1	0.345	0.01	0.904									
De x Si	1	107.482	4.58	<b>0.038</b>									
Mo x De x Si	1	187.870	8.00	<b>0.007</b>									
Error	40	24.052											
<b>(l) <i>B. neritina</i><sup>NS</sup></b>													
				$\ln(x+1)$									
Movement	1	1.094	5.08	0.266									
Depth	1	4.096	1.97	0.394									
Site	1	15.779	61.21	<b>0.000</b>									
Mo x De	1	4.525	1.89	0.400									
Mo x Si	1	0.215	0.84	0.366									
De x Si	1	2.080	8.07	<b>0.007</b>									
Mo x De x Si	1	2.388	9.26	<b>0.004</b>									
Error	40	0.258											
<b>(m) <i>C. nodulosa</i><sup>ES</sup></b>													
				$\ln(x+1)$									
Movement	1	2.642	0.61	0.439									
Depth	1	1.625	0.38	0.543									
Site	1	135.105	31.23	<b>0.000</b>									
Mo x De	1	1.503	0.35	0.559									
Mo x Si	1	2.512	0.58	0.450									
De x Si	1	1.730	0.40	0.531									
Mo x De x Si	1	0.387	0.09	0.766									
Error	40	4.535											

**Bold** indicates significantly different at  $P < 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .

#### 4. Shallow floating structures promote invader dominance

showed an interaction between movement, depth and site (Fig. 4.3 m; Table 4.1). *B. neritina* occupied by far the most space on moving plates at shallow depths, but at one site, occupied more space on fixed plates than on moving (Fig. 4.3 m; Table 4.2). Cover of the exported bryozoan *Celleporaria nodulosa* (Busk 1881) differed between sites, but showed no effect of movement or depth (Fig. 4.3 n, Table 2 m).

##### *Native species recruitment*

Only four native species occupied > 1% space; the mussel *Mytilus galloprovincialis planulatus* (Lamarck 1819), the serpulid *Galeolaria caespitosa* (Mörch 1863), the bryozoan *Fenestrulina mutabilis* (Hastings 1932) and the ascidian *Botrylloides magnicoecum* (Hartmeyer 1912). *M. galloprovincialis planulatus* was the dominant native space occupier and its cover varied significantly with respect to movement, depth and site ( $F_{1,40} = 5.3$ ,  $p = 0.0266$ ). The mussel was more abundant on shallow moving plates at one site and deep moving plates at the other. In contrast, *G. caespitosa* and *F. mutabilis* occupied more space on fixed than moving plates, but this was significant only at one site ( $F_{1,40} = 6.15$ ,  $p = 0.0172$  and  $F_{1,40} = 5.47$ ,  $p = 0.0242$ , respectively). *B. magnicoecum* showed no effect of movement or depth.

## Discussion

This study has demonstrated the combined influences of depth and movement on the early development of marine epifaunal assemblages. As predicted, marine invaders (NIS and ES) were disproportionately more abundant on shallow (0.5 m) moving surfaces than on deep (2 m) moving or fixed surfaces. Moreover, NIS alone were more speciose on all plates, at both depths, than were native species. Effects of movement

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and/or depth were detected for some individual species and in many cases these effects were site-specific, and/or occurred for only fixed or only moving plates.

Various studies have documented differences in recruitment of epifauna at depths much greater than 2 m (e.g. Aleem 1957, Jackson and Winston 1982, Hughes and Jackson 1992), but few have examined depth-related differences in surface waters. Certainly it has been known for a long time that boat hulls tend to be fouled more heavily in the first meter or so of water, but fouling can extend to a depth of a few metres (Visscher 1928). McDougall (1943) noted the luxuriant growth of the invasive bryozoan *Bugula neritina* on floats 15 cm – 1 m deep, and a marked decline in its abundance at greater depths. Similarly, Hurlbut (1991b) found that a colonial ascidian was more abundant 15 cm below the surface on a float than at depths of 1 – 6 m on a fixed piling. But why are many invasive epifauna more prevalent on floating surfaces than just 1.5 m deeper? The most obvious differences between floating structures at the surface versus those 2 m deep are levels of light, turbulence (e.g. due to swash, Holloway and Connell 2002), temperature and salinity. Freshwater layers can persist for days on the surface after heavy rainfall (T.M. Glasby pers. obs.) and different marine fouling species have varying susceptibility to freshwater (Visscher 1928). All these factors are likely to be more variable and extreme at 0.5 m than at 2 m (e.g. increased turbulence around floating docks, Koehl 2007). Thus, the greater proportion of invasive epifauna on shallow moving surfaces might relate to their enhanced ability to withstand physical disturbances; as predicted by the disturbance-invasibility hypothesis (Elton 1958). The potential influences of these physical variables on epifauna are discussed below.

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Several species showed differences in their space occupation with depth, independent of movement. Previous work on pontoons and pilings has highlighted the effect of light availability on these structures as a factor influencing the recruitment of larvae to their surfaces (Glasby 1999b). Light can be expected to decrease with depth and may influence the growth of various algal species; a semi-shaded substrate will favour the competitive exclusion of algae by sessile invertebrates (Glasby 1999b). Differences in recruitment between shallow and deep plates may have been influenced by light availability, particularly larval responses to light. Colonial ascidians have been found to recruit in largest numbers on floating docks, and this is thought to be a result of their photopositive behaviour on release which results in them swimming upwards (Hurlbut 1991a). Similar to the colonial ascidians, the invasive bryozoan *Bugula neritina* is photosensitive during the larval stage, although it becomes indifferent to light prior to metamorphosis (Lynch 1947). In this study, *B. neritina* occupied more space on moving plates at shallow depths (although this was only significant at one site).

Several non-indigenous or exported species occupied more space on moving than on fixed plates at both depths. Their increased percent covers on moving substrates could be in response to stronger water flow over moving surfaces (Perkol-Finkel et al. 2006). Numerous studies have demonstrated that increased water flow can result in increased recruitment of epifauna (e.g. Mullineaux and Garland 1993, Judge and Craig 1997, Leonard et al. 1998). Increased water flow also has the potential to increase food availability and therefore increase size (and aeral coverage) of epifauna (Eckman and Duggins 1993, Perkol-Finkel et al. 2008). In the case of filter-feeding invaders, the ability to settle and remain attached under high shear stresses could advantage them during settlement and transport on boat hulls (see review by Koehl 2007), and may have

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been an important factor in facilitating the spread of invaders on the hulls of ships and their subsequent ability to establish and persist, especially on floating surfaces in ports and estuaries around the world.

In contrast, some invaders including the non-indigenous serpulid *Hydroides elegans* occupied more space on fixed than moving structures, although this did vary between sites. Percent cover of *H. elegans* may have been greater on fixed structures due to the preference of serpulid larvae to settle in areas with reduced water flow. Glasby (2001) found that the cover of *H. elegans* decreased with increasing water flow and Mullineaux and Garland (1991) found that the closely related *H. dianthus* avoided settling in areas with considerable water flow.

While invaders as a group were found to be more abundant on shallow moving surfaces, it is important to acknowledge the variability in the magnitude of effect of movement and depth between sites. Some species displayed effects of movement and/or depth that were consistent across sites, however several species showed localised effects. This variability may be related to the duration of the study and/or to the spatial scale. The recruitment of sessile organisms can vary considerably over small spatial and temporal scales (Sutherland and Karlson 1977, Keough and Butler 1983). Thus, it will be important to repeat the experiment described here in a variety of different locations to test for the generality of these results. The identity of species in any fouling assemblage will of course differ among locations and times, but the main question to answer is whether invasive epifauna in general, or particular wide-spread or problematic invaders, are consistently most abundant on shallow moving surfaces and more abundant than native epifauna. Arguably a study of longer duration might have lead to the results

#### 4. Shallow floating structures promote invader dominance

being more spatially consistent, but Butler and Connolly (1999) found overall assemblage composition on pier pilings had not converged to a 'stable' state even after 6.5yr.

Notably, some invaders showed opposite responses to fixed and moving treatments including the exported barnacle *Balanus trigonus* and non-indigenous bryozoan *Microporella umbracula* (more on fixed) compared to the non-indigenous ascidian *Diplosoma listerianum* and exported ascidian *Herdmania momus* (more on moving). Native species as a group showed no effect of movement or depth, however individually they showed varying responses to fixed and moving plates. The dominant space occupier *Mytilus galloprovincialis planulatus* occupied more space on moving plates and in most cases this was regardless of depth. For the purposes of this study *M. galloprovincialis planulatus* has been classified as native (according to Daguin and Borsa 2000, Hilbish et al. 2000, Beu 2004), however this genus is currently under genetic review and it is possible that the species is exported. Its abundance on moving structures would make it a likely candidate for transport on vessel hulls and establishment on pontoons in other countries.

### Conclusion

A good understanding of the ecological effects of adding artificial structures to the coastal environment is essential for informing management strategies. The construction of boating structures creates novel habitat and epibiotic assemblages have been shown to develop differently on these structures compared to nearby natural rocky reef (Connell 2001, Knott et al. 2004, Glasby et al. 2007). This study has demonstrated the potential for shallow moving surfaces to promote invader dominance and goes some



#### *4. Shallow floating structures promote invader dominance*

way to explaining why moving structures such as vessel hulls (particularly sections close to the water's surface), pontoons and navigation markers are 'hotspots' for invaders. This adds to a growing body of findings related to invasibility; including that invaders are more abundant on concrete surfaces (Glasby et al. 2007), on copper treated surfaces and in areas with high levels of copper contamination (Dafforn et al. 2008, Piola and Johnston 2008a, Dafforn et al. 2009a). Future research should consider multifactorial experiments to tease apart the relative contributions of all these factors in enhancing invasibility.

## Chapter 5

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### **Sedimentation and native species enhance resistance to marine invasion in subtidal rocky reef assemblages.**

#### **Abstract.**

The invasibility of a system is thought to be strongly linked to local biotic and abiotic conditions acting as selective filters on arriving non-indigenous species (NIS). Subtidal rocky reefs tend to be dominated by a resident community of spatially competitive turf or canopy-forming macroalgae that provide a barrier to arriving invasive propagules requiring bare substrate for settlement. While some NIS have invaded rocky reef habitats, generally they are more abundant on artificial structures such as pilings or pontoons. Such structures present large areas of vertical substrata that are relatively shaded and as a result have a reduced algal presence.

We surveyed hard-substrate assemblages in situ on pilings, pontoons and rocky reef in Sydney Harbour. NIS were more speciose on piers and pilings relative to rocky reef while native species richness was similar across structures. A restricted set of non-indigenous invertebrates and algae were also found on the reef. Most NIS were invertebrates, whereas many of the native species were algae. We tested the hypothesis that the ability to invade natural rocky reef is influenced by the combined effects of resource patch size and resident assemblage using experimental clearings on reefs. We also tested the effect of resident assemblage, shading and orientation on invasion using sandstone settlement plates. Patch size and shading did not affect colonisation of reef clearings or sandstone plates by NIS, however shading reduced the abundance of native algae on sandstone plates. NIS were advantaged by the availability of bare space and inhibited by high levels of sedimentation on horizontal plates. Increased anthropogenic

modification of estuarine habitats may release resources (e.g. space) and lead to increased opportunities for NIS to gain a foothold in assemblages on reef flats and potentially facilitate other invaders.

## **Introduction**

Habitat modification and invasive species are widely acknowledged to be primary factors in the loss of global biodiversity (Wilcove et al. 1998, Bax et al. 2003, Molnar et al. 2008). It has become increasingly apparent that research efforts should focus on the interactive effects of these factors, rather than considering them in isolation (Ruiz et al. 1999, Didham et al. 2007). In marine and terrestrial invasions, loss or modification of habitat can result in reduced native diversity and create windows of opportunity for non-indigenous species (NIS) (Vitousek et al. 1997, Levine 2000). The successful establishment of a species upon introduction into a new environment will depend on many features intrinsic to the organism, including its growth and reproductive rates and ability to evade native predators and/or parasites (Byers 2002b, Bishop and Peterson 2006). Successful establishment is also linked to the receiving community's susceptibility to invasion, which is generally considered to be greatest in disturbed environments, and least in diverse communities where resources are limited and all available niches may be occupied (Davis et al. 2000, Levine 2000, Stachowicz et al. 2002a).

The construction of artificial structures in marine and terrestrial environments provides additional and often novel habitat. Artificial structures constructed in marine environments (e.g. pilings, pontoons and seawalls) create islands of hard-substrate and these structures can change local hydrodynamic conditions which may alter the rate at

which nutrients, organic material, larvae and spores are transported (Koehl 2007). The increasing intensity of storms and rising sea levels associated with climate change are likely to increase the need for artificial structures in coastal defences (Nicholls and Mimura 1998, FitzGerald et al. 2008). Therefore it is important to understand the ecological implications of these anthropogenic modifications to ports and harbours.

The biota on pilings, pontoons and seawalls have been well described and these habitats support distinct assemblages from natural rocky reefs (Connell and Glasby 1999) (Connell and Glasby 1999, Glasby 1999a, Knott et al. 2004). Pontoons are most similar to pilings and both are more diverse than seawalls or natural reefs (Glasby and Connell 1999), being characterised by invertebrates such as encrusting bryozoans, serpulid polychaetes, sponges and ascidians. In contrast, reefs are often dominated by spirorbid polychaetes and filamentous and foliose algae (Glasby 1999a). The abundance of artificial structures such as pilings, pontoons and seawalls in ports and harbours has the potential to provide hard substratum for fouling species arriving on vessel hulls to settle and establish reproducing populations (Carlton 1987, Ruiz et al. 1997, Bulleri and Airolidi 2005, Glasby et al. 2007). Recent research suggests that artificial structures may in fact act as ‘stepping stones’ (Glasby and Connell 1999) or ‘corridors’ (Bulleri and Airolidi 2005) for the spread of NIS into natural communities.

Association with artificial structures is considered an identifying characteristic of NIS (Chapman and Carlton 1991). We know that substrate composition has the potential to affect the colonisation of NIS and indeed many native epibiota (Anderson and Underwood 1994, Bourget et al. 1994, Lapointe and Bourget 1999, Glasby 2000). Movement and proximity to surface are also important factors in NIS recruitment, with

invaders tending to favour shallow floating structures such as pontoons (Glasby et al. 2007, Dafforn et al. 2009b). In fact, many NIS arriving on ship hulls are confined to artificial substrata (Paulay et al. 2002) with few able to invade natural communities (but see Crooks 1998, O'Brien et al. 2006, Osman and Whitlatch 2007). It could be argued that while NIS may remain confined to artificial structures, they pose only an economic risk to commercial practices that rely on artificial structures [fouling of industrial pipes e.g. *Dreissena polymorpha* (Ludyanskiy et al. 1993, Pimentel et al. 2000) and *Mytilopsis sallei* (Rao et al. 1989) and damage to port infrastructure e.g. *Teredo navalis* (Cohen and Carlton 1995)]. In this case the primary ecological risk occurs when NIS are able to invade natural systems (Willan 1987, Crooks 1998, Reusch and Williams 1998, O'Brien et al. 2006), and NIS that have this characteristic should be identified and prioritised for pest management.

In natural systems, the resident community may be thought of as the front line of defense against invading NIS. More diverse communities are thought to enhance invasion resistance because competition for resources in the community is greater, with fewer left available for invading species (Elton 1958, Tilman 1997). Space is a primary limiting resource in marine hard-substrate communities (Jackson 1977). On horizontal rocky reefs, macroalgae tend to dominate space with the result being that most invertebrates are limited to vertical walls and shaded substrates (Paine 1990, Baynes 1999, Knott et al. 2004). Since many marine invaders are sessile invertebrates, it is likely that they are being excluded from the reef by the pre-emptive space occupation by algae (but see Connell 2003). Theory and past experimental evidence would suggest that for these invaders to gain a foothold in rocky reefs there needs to be a release of

resources, in this instance space (Davis et al. 2000, Stachowicz et al. 2002a, Altman and Whitlatch 2007).

Anthropogenic disturbance in estuaries plays an important role in opening up space for invading species by physical removal of the resident assemblage (e.g. scour from vessel anchoring and swash). These disturbances create nonisolated clearings because they are surrounded by the resident algal assemblage (Keough 1984). Where clearings occur they are likely to be quickly re-colonised by vegetative growth of the surrounding algae and invertebrate recruits overgrown (Keough 1984, Connell and Keough 1985).

Therefore if an invading species was able to take advantage of a temporary clearing, it would also need to resist overgrowth by encroaching algal species. Patch size plays a role in the outcome of these interactions, with larger patch sizes providing invading larval recruits and solitary organisms more time to become large enough to resist overgrowth (Connell and Keough 1985).

Environmental conditions on the reef (including light and sediment load) are likely to contribute to sustained macroalgal dominance (Airoidi 1998, Glasby 1999c, Irving and Connell 2002, Airoidi 2003). Horizontal surfaces facing upwards will be exposed to greater light intensity and accumulation of settling sediments, while vertical surfaces and horizontal surfaces facing downwards will receive little light and accumulate less sediment. Higher sediment levels on horizontal substrates can also result in problems for filter-feeding invertebrates by smothering new recruits or clogging filter feeding apparatus (Ostroumov 2005), but macroalgae, particularly turfs, are highly resistant to sedimentation (Airoidi 1998, 2003). Light is also thought to be a primary factor in the ability of algae to monopolize space on horizontal rocky reefs (Baynes 1999). In

contrast, more shaded vertical surfaces of artificial and natural structures are often dominated by invertebrates (Knott et al. 2004). Experimental shading of communities on rocky reefs can shift this competitive interaction and result in the development of invertebrate assemblages that are more similar to those found on pilings (Glasby 1999b, Miller and Etter 2008). Because many invaders are sessile invertebrates, shading has the potential to facilitate invasion, by reducing light and sediment levels and creating conditions that can be exploited.

Artificial structures typically have shaded vertical surfaces or horizontal surfaces facing downwards which create ideal conditions for recruiting sessile invertebrates. In addition, many artificial structures are found in close proximity to vessels and may provide a settlement surface for propagules from NIS arriving on vessel hulls. Here we compare the distribution of NIS between artificial structures (pilings and pontoons) and natural rocky reefs using underwater surveys. We hypothesised that NIS would be more abundant on artificial structures, particularly pontoons (Glasby et al. 2007, Dafforn et al. 2009b), than natural rocky reefs. We identified a sub-set of reef invaders from the total pool of NIS sampled in the survey and created experimental clearings on the reef to test for effects of resident assemblage and patch size on the invasibility of reef communities. We also deployed sandstone plates as a proxy for rocky reef to experimentally test the effects of light, orientation and resident assemblages on invasibility. We formulated several hypotheses, firstly that NIS would be less able to invade established than new assemblages in both experimental clearings and sandstone plates. Secondly that small, cleared patches would be rapidly re-colonised by algae, and would therefore be less invaded than larger or uncleared patches. We also hypothesised that NIS would invade vertical shaded areas more than horizontal, unshaded areas due

to reduced light and sedimentation, and the resultant reduction in competition from algae.

## Methods

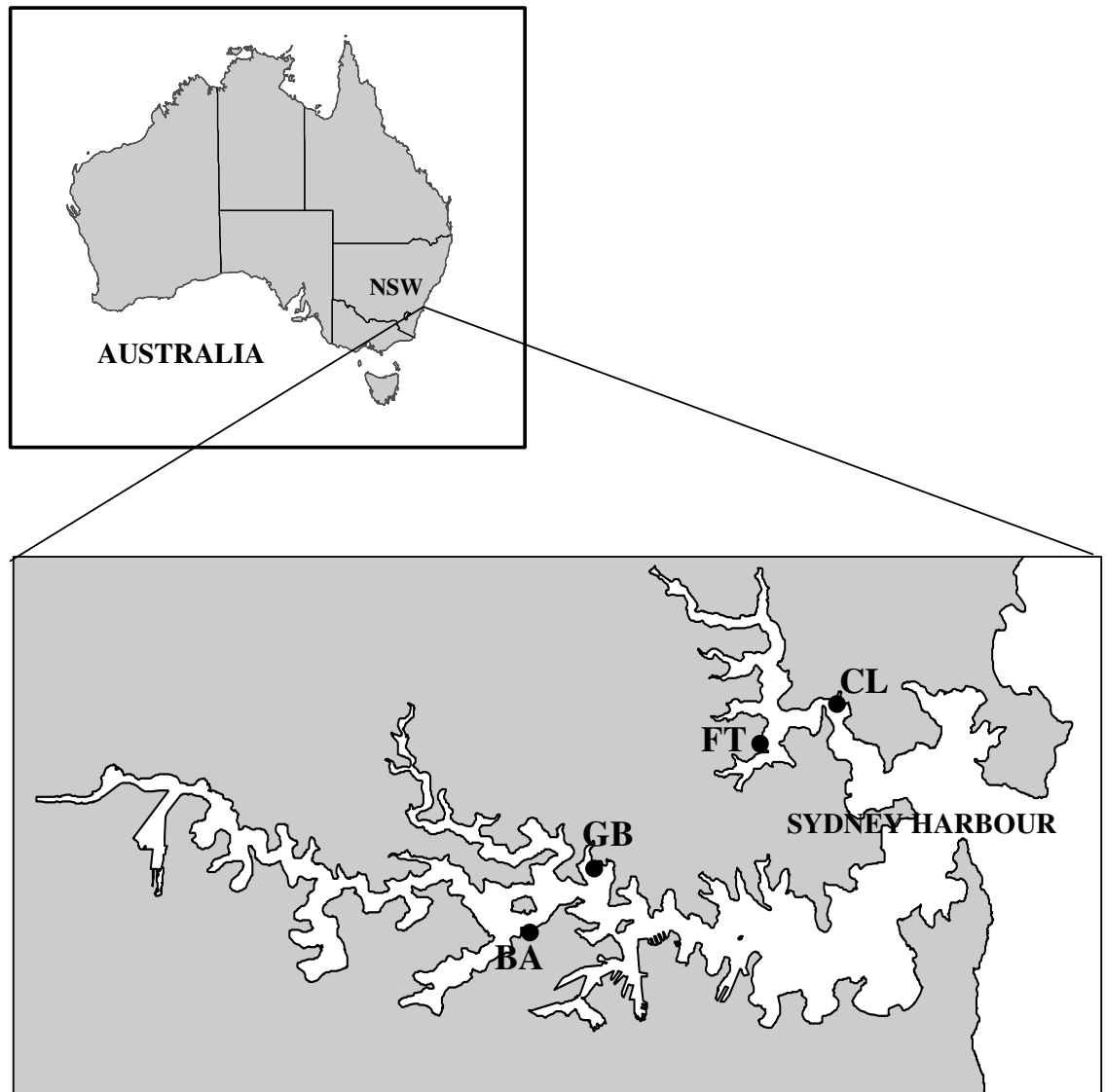
### *Study sites*

The study was conducted at four subtidal sites within Port Jackson on the southeast coast of Australia (33°50' South 151°22' East; Fig. 5.1) for a period of 32 months from April 2006 to December 2008. Port Jackson is a highly urbanised estuary and its shores are lined with artificial structures interspersed with sandstone rocky reef outcrops. The sites were located in sheltered embayments within the harbour, protected from oceanic swells, and experiments were conducted between 1 – 3 m below mean low water springs (MLWS). The lowest subtidal area of rocky reef (~ 0 – 2 m) is dominated by turfing algae (primarily *Corallina officinalis*) whilst an area of canopy-forming algae (*Ecklonia radiata* and *Sargassum* sp.) occurs between 2-5 m. Below this are soft sediments.

### *Non-indigenous species on artificial and natural structures*

We compared distributions of non-indigenous and native fouling species on artificial (pilings and pontoons) and natural (rocky reefs) structures at four sites in Port Jackson (structures were within 50 m; Fig. 5.1). Pilings and pontoons were located at marinas and were constructed of wood (all pilings), concrete (pontoon – Balmain), fibreglass (pontoon – Clontarf, Gore Bay) and plastic (pontoon - Fig Tree). Information regarding the age and time since last cleaning were not available. Non-disruptive sampling was conducted during the southern hemisphere winters of 2006 and 2007. Fifteen 0.25 m<sup>2</sup> quadrat samples were randomly taken from the surfaces of pier pilings, pontoons and





**Figure 5.1.** Study sites for *in-situ* underwater survey (BA, FT, GB and CL), rocky reef clearings (BA, FT and GB) and sandstone plate deployment (BA and FT). BA = Balmain, FT = Fig Tree, GB = Gore Bay and CL = Clontarf.

rocky reefs between 0 – 1 m below MLWS. Vertical surfaces were sampled in all cases as pilings and pontoons have no upward facing horizontal surfaces that would be comparable to horizontal rocky reef. Species percent cover in each quadrat was estimated using a grid of 36 regularly spaced points. Organisms within the quadrat, but not under a point were given a nominal value of 0.5%.

*Effect of resident community and patch size on invasibility of horizontal rocky reefs*

In situ sampling (described above) found several NIS present on rocky reefs. We established experimental clearings on outcrops of rocky reef to test resident community resistance to invasion and the effect of patch size on invasion. Vertical substrate was limited at these sites so to maximise replication, clearings were restricted to horizontal substrate. Treatments were (1) resident assemblage, with two levels (established and new) and (2) patch size, with three levels (0.05 m<sup>2</sup>, 0.1 m<sup>2</sup> and 0.15 m<sup>2</sup>). These treatments were orthogonally combined to give six treatment combinations, each with six replicates at three sites in Port Jackson (Fig. 5.1). Clearings of 0.05 m<sup>2</sup>, 0.1 m<sup>2</sup> and 0.15 m<sup>2</sup> were randomly marked out with 0.5m<sup>2</sup> spacing on a 20 m<sup>2</sup> area of sandstone reef at each site. Clearings were at 0.5 – 1 m below MLWS and were either cleared of all growth or unmanipulated. ‘Established’ assemblages were clearings that were not disturbed and all space was occupied by turfing algae (primarily *Corallina officinalis*). ‘New’ assemblages were clearings that had been scraped leaving bare sandstone substrate. Five groups of replicate clearings were created at ~ 2 mo intervals between December 2007 and August 2008 and each set were photographed after eight weeks. NIS were identified from the photos.

*Effects of sedimentation, shading and biotic resistance on invasibility*

To further investigate factors affecting the invasibility of rocky reefs we conducted a manipulative experiment using Hawkesbury sandstone settlement plates (0.15 m<sup>2</sup>, 2cm thick) as this is the primary hard substrate found in Port Jackson. Treatments were (1) resident assemblage, with two levels (established and new), (2) shading, with three levels (shaded, shade control and unshaded control) and (3) orientation, with two levels (horizontal and vertical). These treatments were orthogonally combined to give 12 treatment combinations, each with five replicates. 'Established' assemblages were those on settlement plates that had previously been deployed subtidally for 12 months and these typically comprised between 60 - 90% cover of the brown alga *Sargassum* sp. (this species is widespread throughout the Sydney region and not classified as non-indigenous). 'New' assemblages were bare sandstone plates that had not been in seawater and were lacking a biofilm. Biofilms play an important role in invertebrate recruitment (Zobell and Allen 1935, Zardus et al. 2008) (although these can be extensive within 24 h and would have been unlikely to have a significant effect on our comparison between new and established assemblages which was conducted over 8 months). Shaded plates had black Perspex roofs (35 x 45 cm with a 3 cm folded edge). Shade control plates had transparent Perspex roofs, to distinguish any differences in sedimentation and water flow that might result from the presence of the roofs. Roofs were cleaned weekly to remove fouling.

Perspex roofs were fastened to the angles 30 cm above the plates. Frames were weighted to the sea floor at between 1-2 m below MLWS on rocky reefs at two sites in Port Jackson (Fig. 5.1). Experiments were deployed in November 2007 and retrieved in

July 2008. Plates were collected and *Sargassum* sp. canopy was removed from the plates to census the understorey organisms. The algae was then dried in an oven at 60°C for 24 h and weighed. The upward (horizontal) or outward (vertical) facing surfaces were sampled live by placing a grid of 100 points over the surface to assess percent covers of different species. Organisms on the plate, but not under a point were given a nominal value of 0.5%.

Additional environmental variables were measured to test for artefacts in the results from using roofs. Midday light was measured above two replicate plates of each light treatment at each site for 28 days in December 2007 using a HOBO<sup>®</sup> temperature/light data logger (Model UA-002-08; Onset Computer Corporation). Roofs were also cleaned weekly to remove sediment and fouling and ensure the effectiveness of the shade control treatment. Four replicate sediment traps (300 mm tall plastic cylinders with a diameter of 45 mm) were attached vertically to a 10 kg weight and deployed adjacent to the aluminium frames at each site for 28 d. A comparative measure of sediment loads on each plate was also taken using a syringe to vacuum 60 ml of fine sediment from a 1 x 1 cm area in the corner of two replicate plates in each treatment. In the lab, each sample was transferred into a container of known weight and left to settle for 24 h to obtain the coarse fraction. The water was then suctioned off the top and filtered through a dried and pre-weighed 1µm filter paper to obtain the fine fraction. Coarse and fine fractions were oven-dried at 70°C and weighed. Temperature, salinity and turbidity measurements were also collected weekly during the eight month deployment.

#### *Data analyses*

Species identified during this study were classified as non-indigenous or native according to the literature (see Appendix 1). Algae were classified as native following the reasoning of Chapman and Charlton (1991) apart from the brown algae *Colpomenia sinuosa* and *Dictyota dichotoma* which are non-indigenous to Australia (Glasby et al. 2007). Statistical analyses were conducted on non-indigenous and native species as a group as well as for individual NIS occupying > 0.5% space using WinGmav5. All data were assessed for normality and homogeneity of variance using residual frequency histograms and Cochran's C test, respectively.  $\ln(x + 1)$  or  $\sqrt{x + 1}$  transformations were used to obtain homogeneous variances where necessary. Where transformed data remained heterogeneous, the untransformed data were used for analysis and  $\alpha$  was set at 0.01 for the ANOVA (Underwood 1981). Post hoc pooling of non-significant terms ( $P < 0.25$ ) was used to increase the power of statistical tests (Underwood 1981).

Non-indigenous and native species richness data from the in-situ survey were analysed with a three-way ANOVA that treated structure (piling, pontoon, reef) and time (2006, 2007) as fixed and site (Balmain, Fig Tree, Gore Bay, Clontarf) as random effects. Individual species percent covers were variable and only those present on all structures could be analysed with ANOVA. Results are presented in text and full tables are given in Appendix VI.

Reef plot invasion data were analysed with a four-way ANOVA that treated assemblage (new, established), patch size (0.05 m<sup>2</sup>, 0.1 m<sup>2</sup>, 0.15 m<sup>2</sup>) and sampling time (December 2007, February 2008, April 2008, June 2008 and August 2008) as fixed effects. The number of clearings invaded by non-indigenous invertebrates and algae were compared

as well as all invading species. Native invertebrates were few and only present in established assemblages so were excluded from the analyses. ANOVA models were adjusted to fit the data in each case, e.g. *Styela plicata* only invaded established assemblages so the factor ‘assemblage’ was removed from the analysis.

Non-indigenous and native species percent cover on sandstone plates was analysed with a four-way ANOVA that considered resident assemblage (established, new), shading (shaded, shade control, unshaded control), substrate orientation (horizontal, vertical) as fixed effects and site (Balmain, Fig Tree) as random. ANOVA models were adjusted to fit the data in each case, e.g. *Diplosoma listerianum* only occurred on vertical plates and so the factor ‘orientation’ was removed from the analysis. Algae were the dominant native species comprising ~30% (Balmain) and ~80% (Fig Tree) of native occupied space and therefore we also analysed percent cover of all native algae and all native invertebrates to assess any differential effects of the treatment combinations. Non-indigenous algae were comparatively absent (comprising less than 0.5% of non-indigenous occupied space) and are therefore not considered in a separate analysis. Dry weight sediment and *Sargassum* sp. data collected from the plates were analysed using a four-way ANOVA (details above). Turbidity, sediment and shading were also compared between sites.

## Results

### *Non-indigenous species on artificial and natural structures*

Fifty-two taxa were sampled during the in situ survey (most to genus or species). These included species of encrusting and arborescent bryozoans, acorn barnacles, solitary and colonial ascidians, and serpulid polychaetes. Nineteen species were identified as non-

indigenous, twenty-three as native and ten as cryptogenic (Carlton 1996a) (see Appendix II for a detailed species list and classifications). As a group cryptogenic species occupied an average of < 0.5 % cover so were excluded from further analyses.

Non-indigenous and native species richness varied between artificial and natural structures and also showed spatial and temporal variation (Fig. 5.2; Table 5.1). NIS richness was up to four times greater on artificial (pilings and/or pontoons) than natural structures (Fig. 5.2 a; SNK  $p < 0.05$ ) and generally showed no difference between pilings and pontoons (2006: at three out of four sites and 2007: at two out of four sites; SNK  $p > 0.05$ ). Native species richness tended to be greater on reefs (2006: at three out of four sites; SNK  $p < 0.05$ ) or similar on all structures (2007: at three out of four sites; SNK  $p > 0.05$ ) (Fig. 5.2 b).

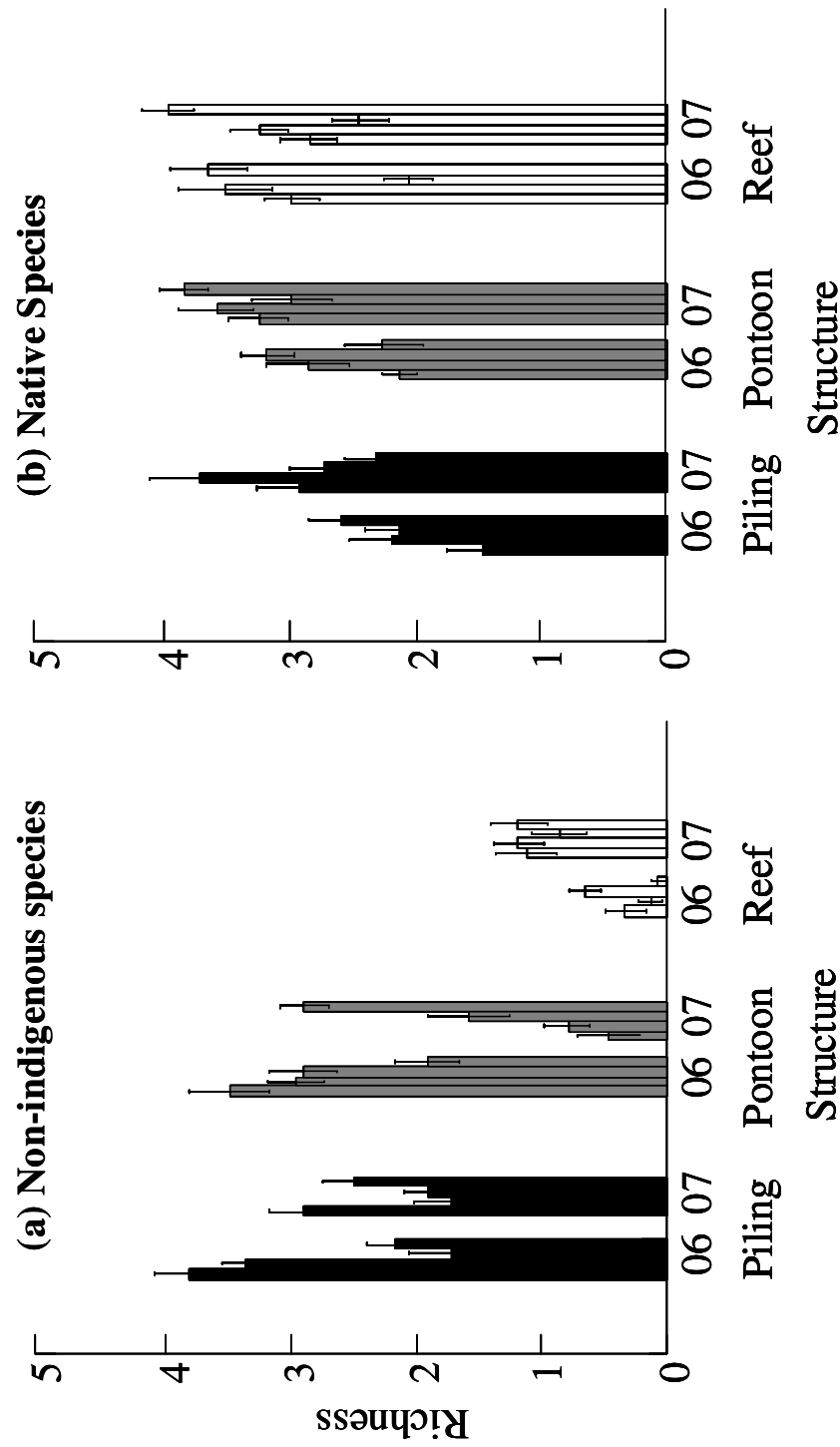
Percent covers of other species varied between structures and were spatially and temporally variable. The arborescent bryozoan *Bugula neritina* only occurred on artificial structures (Fig. 5.3 a) while, the encrusting bryozoan *Watersipora arcuata* was only present on reef and absent from artificial structures (Fig. 5.3 b). Seven other NIS were present on the reef, but occupied most space on artificial structures, including the encrusting bryozoans *Schizoporella errata*, *Cryptosula pallasiana* and *Watersipora subtorquata*, the arborescent bryozoan *Tricellaria inopinata*, the solitary ascidian *Styela plicata*, the colonial ascidian *Botrylloides leachi* and the alga *Dictyota dichotoma*. *S. errata* was most abundant on pilings and absent from reefs (Fig. 5.3 c). *C. pallasiana* and *B. leachi* were also more abundant on pilings or pontoons and largely absent from reef (Fig. 5.3 d - e; *C. pallasiana*:  $F_{6,336} = 28.77$ ,  $p = 0.000$ , *B. leachi*:  $F_{6,336} = 9.93$ ,  $p = 0.000$ ) although this was only significant for one site (*C. pallasiana* - 2006 and *B. leachi*

- 2006/7; SNK  $p < 0.05$ ). *W. subtorquata* occupied most space on pilings or pontoons (Fig. 5.3 f;  $F_{6,336} = 5.82$ ,  $p = 0.000$ ) but this was only significant at one site (2006/7; SNK  $p < 0.05$ ). *T. inopinata* and *S. plicata* occupied more space on artificial structures than reef (Fig. 5.3 g – h; *T. inopinata*:  $F_{6,336} = 3.80$ ,  $p = 0.001$ , *S. plicata*:  $F_{6,336} = 9.47$ ,  $p = 0.001$ ), but did not differ between pilings or pontoons at one (2006) or two (2007) sites (*T. inopinata* – 2007 and *S. plicata* – 2006/7; SNK  $p < 0.05$ ). The brown alga *D. dichotoma* was also more abundant on artificial structures than reef (Fig. 5.3 i; *D. dichotoma*:  $F_{6,336} = 10.44$ ,  $p = 0.001$ ) and occupied more space on pilings than pontoons at one (2006) or two (2007) sites (SNK  $p < 0.05$ ). In contrast, the brown alga *Colpomenia sinuosa* occupied more space on reef and was largely absent from artificial structures, only occurring on pontoons at one site in 2007 (Fig. 5.3 j). The serpulid *Hydroides elegans* was patchily distributed and occupied most space on pontoons at one site in 2006 (Fig. 5.3 k).

#### *Effect of resident community and patch size on invasibility of rocky reefs*

Non-indigenous invertebrates invaded more new than established assemblages on the reef, although this was variable between sampling times (most clearings invaded in December 07 and April 08; SNK  $p < 0.05$ ) and there was no effect of patch size on invasibility (Fig. 5.4 a; Table 5.2). Non-indigenous algal invasion also differed between sampling times (Fig. 5.4 b; Table 5.2) although there was a generally linear increase from December 07 to August 08, with the most clearings invaded in July and August 08 (SNK  $p < 0.05$ ). Assemblage and patch size had no effect on algal invasion (Fig. 5.4 b; Table 5.2).





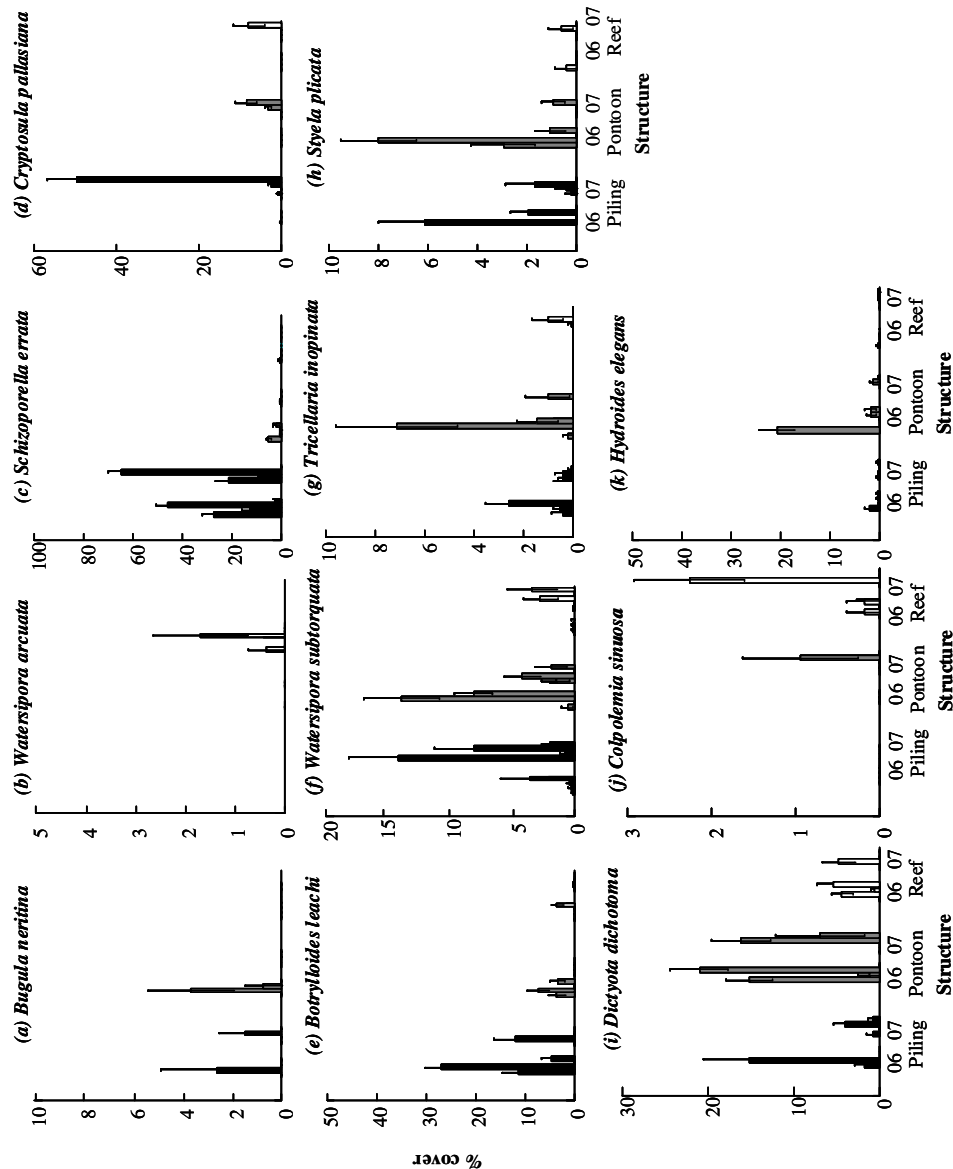
**Figure 5.2.** Species richness (+ S.E.) of native and NIS sampled underwater on pilings, pontoons and rocky reefs in 2006 and 2007. Individual bars represent sites ordered Balmain, Fig Tree, Gore Bay and Clontarf from left to right.

**Table 5.1.** Analyses of variance comparing the diversity (richness) of non-indigenous and native species on pilings, pontoons and rocky reefs at four sites in Port Jackson in 2006 and 2007.

Species Richness		(a) Non-indigenous Species			(b) Native Species		
Source	DF	MS	F	P	MS	F	P
Structure	2	112.95	19.45	<b>0.002</b>	12.31	2.79	0.139
Site	3	2.98	3.78	<b>0.011</b>	9.35	8.70	<b>0.000</b>
Time	1	12.47	0.75	0.451	30.04	24.29	<b>0.016</b>
St x Si	6	5.81	7.36	<b>0.000</b>	4.42	4.11	<b>0.001</b>
St x Ti	2	36.77	5.09	0.051	5.50	1.17	0.371
Si x Ti	3	16.71	21.18	<b>0.000</b>	1.24	1.15	0.329
St x Si x Ti	6	7.22	9.15	<b>0.000</b>	4.68	4.36	<b>0.000</b>
Error	336	0.79			1.07		

**Bold** indicates significantly different ( $P < 0.05$ ).

Six NIS invaded the experimental reef clearings (*Styela plicata*, *Watersipora arcuata*, *Hydroides elegans*, *Diplosoma listerianum*, *Dictyota dichotoma* and *Colpomenia sinuosa*) and all except *D. listerianum* had previously been sampled on the reef. The serpulid *H. elegans* only invaded new assemblages at one sampling time (Fig 5.4 c). The solitary ascidian *S. plicata* was only able to invade established assemblages, while the colonial ascidian *D. listerianum* only invaded new assemblages (Fig. 5.4 d – e; Table 5.3). The encrusting bryozoan *W. arcuata* consistently invaded more new assemblages than established ones (Fig. 5.4 f; Table 5.4). *W. arcuata* invasion was variable between patch size (greatest in 0.15 m<sup>2</sup>; SNK  $< 0.05$ ) and over time (greatest in December 07, April 08 and August 08; SNK  $p < 0.05$ ). Patch size had no detectable effect on invasibility of the clearings for other non-indigenous invertebrates or algae. Invasion of clearings by the brown algae *D. dichotoma* and *C. sinuosa* did not differ between new and established assemblages and appeared to increase between December



**Figure 5.3.** Percent cover (+ S.E.) of non-indigenous species sampled underwater on pilings, pontoons and rocky reefs in 2006 and 2007. Individual bars represent sites ordered Balmain, Fig Tree, Gore Bay and Clontarf from left to right.

07 and August 08 (Fig. 5.4 g - h; Table 5.4), however this pattern was only significant for *C. sinuosa* (SNK  $p < 0.05$ ).

*Effects of sedimentation, shading and biotic resistance on invasibility*

Sixty-two taxa were sampled during the sandstone plate experiment (most to genus or species). Eighteen species were identified as non-indigenous, twenty-one as native and twenty-one as cryptogenic. As a group cryptogenic species occupied an average of  $< 0.8\%$  cover so were excluded from further analyses. Patterns of non-indigenous and native species cover showed a similar response to treatments at both sites, although cover at Balmain was approximately twice that of Fig Tree resulting in site effects (Fig. 5.5). Resident assemblage and orientation were important factors influencing space occupation by non-indigenous and native species. NIS always occupied most space in new assemblages and on vertical plates ( $F_{1,106} = 13.84$ ,  $p = 0.000$ ; SNK  $p < 0.05$ ), but did not differ between shading treatments (Fig. 5.5). Cover of native species was greatest in established assemblages and on vertical plates ( $F_{2,104} = 14.29$ ,  $p = 0.000$ ; SNK  $p < 0.05$ ). Cover of native species was also affected by the shading treatments and was greatest on horizontal unshaded plates or vertical shaded plates ( $F_{2,104} = 3.24$ ,  $p = 0.043$ ; SNK  $p < 0.05$ ).

Invertebrates comprised  $> 90\%$  of NIS, while native species were mostly algae (Fig. 5.5). Native invertebrates and algae differed in their responses to the experimental treatments and the magnitude of effect was often greater at one site than the other. Native invertebrates always occupied more space on vertical compared to horizontal ( $F_{1,102} = 308.94$ ,  $p = 0.000$ ; SNK  $p < 0.05$ ), and in new compared to established assemblages at one site ( $F_{1,102} = 8.52$ ,  $p = 0.004$ ; SNK  $p < 0.05$ ). In comparison, native

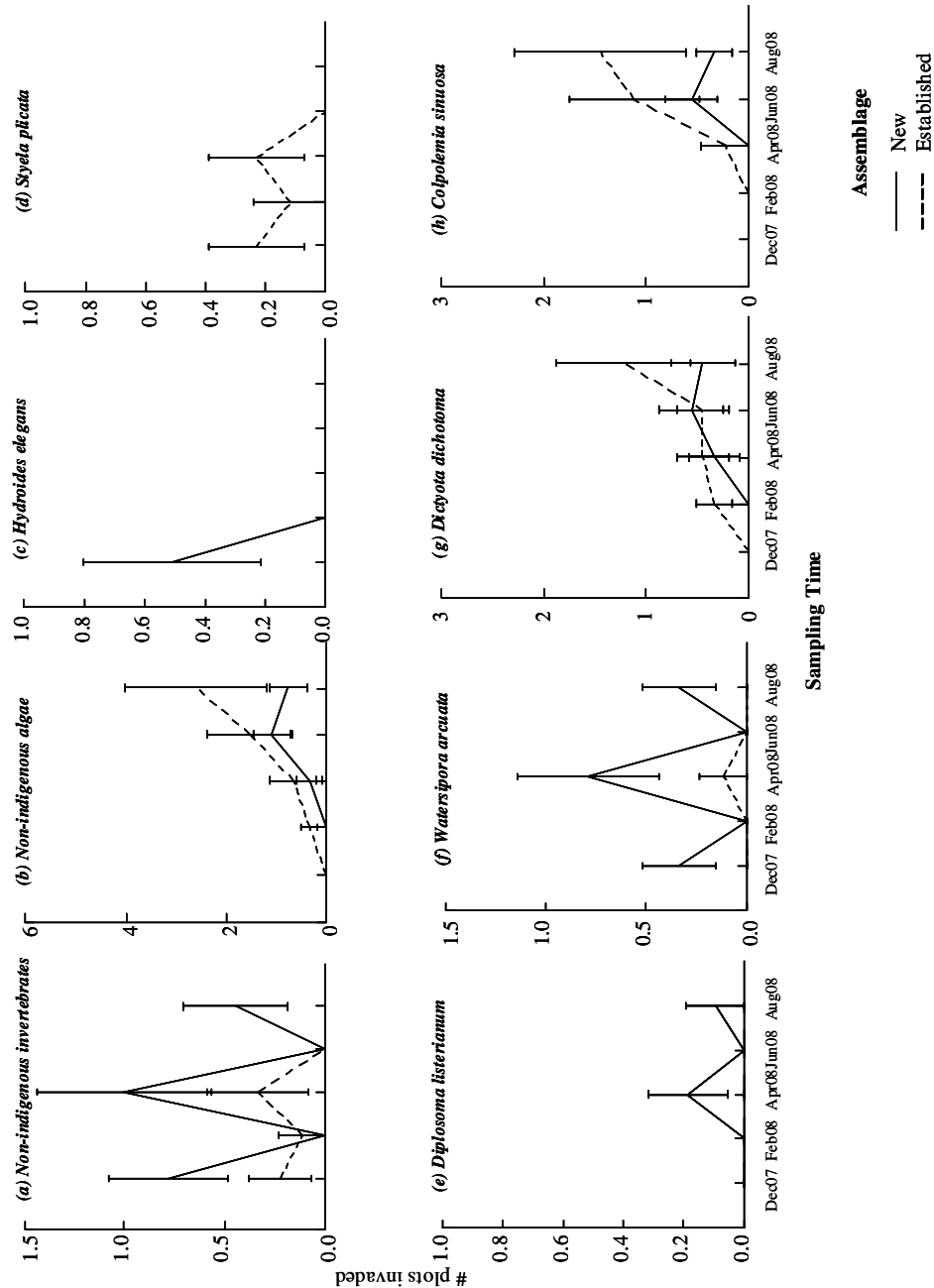
**Table 5.2.** Analyses of variance comparing the number of rocky reef clearings invaded by (a) non-indigenous invertebrates and (b) non-indigenous algae at four sites in Port Jackson over five sampling times. Clearings contained new or established assemblages and were of size 0.05, 0.10 or 0.15 m<sup>2</sup>.

# clearings invaded	(a)Non-indigenous invertebrates*				(b) Non-indigenous algae*		
Source	DF	MS	F	P	MS	F	P
Assemblage	1	0.74	5.23	<b>0.026</b>	31.21	1.02	0.317
Patch size	2	0.11	2.42	0.097	25.14	0.58	0.564
Sampling Time	4	0.33	3.84	<b>0.007</b>	3.66	3.65	<b>0.010</b>
As x Pa	2	0.15	0.99	0.379	33.68	0.19	0.827
As x Ti	4	0.18	1.36	0.260	4.99	0.33	0.856
Pa x Ti	8	0.14	0.53	0.828	3.67	0.13	0.998
As x Pa x Ti	8	0.17	0.26	0.976	2.96	0.20	0.990
Error	60	0.11			0.71		

**Bold** indicates significantly different ( $P < 0.05$ ). \* indicates  $\ln(X + 1)$  transformation to obtain

homogeneous variances.

5. Invasibility of rocky reef assemblages



**Figure 5.4.** Number of new and established clearings (+ S.E.) invaded by non-indigenous invertebrates and algae at five sampling times between December 2007 and August 2008. Data are pooled across patch sizes and sites.

**Table 5.3.** Reduced model analyses of variance comparing the number of rocky reef clearings invaded by individual species at four sites in Port Jackson over five sampling times. Clearings were of size 0.05, 0.1 or 0.15 m<sup>2</sup>.

# plots invaded	(a) <i>Styela plicata</i>				(b) <i>Diplosoma listerianum</i>		
Source	DF	MS	F	P	MS	F	P
Patch size	2	0.16	1.40	0.262	0.07	1.00	0.380
Sampling Time	4	0.11	1.00	0.423	0.09	1.33	0.280
Pa x Ti	8	0.04	0.40	0.912	0.04	0.58	0.783
Error	30	0.11			0.07		

**Table 5.4.** Analyses of variance comparing the number of rocky reef clearings invaded by individual species at four sites in Port Jackson over five sampling times. Clearings contained new or established assemblages and were of size 0.05, 0.1 or 0.15 m<sup>2</sup>.

	(a) <i>Watersipora arcuata</i> *				(b) <i>Dictyota dichotoma</i> *			(c) <i>Copomenia sinuosa</i> *		
Source	DF	MS	F	P	MS	F	P	MS	F	P
Assemblage	1	0.62	10.49	<b>0.002</b>	0.25	1.19	0.279	0.31	1.60	0.211
Patch size	2	0.19	3.16	<b>0.049</b>	0.05	0.23	0.795	0.14	0.72	0.489
Sampling Time	4	0.21	3.53	<b>0.012</b>	0.40	1.90	0.122	0.82	4.26	<b>0.004</b>
As x Pa	2	0.08	1.39	0.258	0.04	0.17	0.844	0.08	0.42	0.656
As x Ti	4	0.12	1.98	0.109	0.09	0.41	0.804	0.09	0.44	0.776
Pa x Ti	8	0.05	0.86	0.556	0.05	0.21	0.987	0.07	0.39	0.924
As x Pa x Ti	8	0.03	0.45	0.886	0.07	0.33	0.949	0.03	0.16	0.995
Error	60	0.06			0.21			0.19		

**Bold** indicates significantly different ( $P < 0.05$ ). \* indicates  $\ln(X + 1)$  transformation to obtain

homogeneous variances.

algae occupied more space in established than new plates (Fig. 5.5;  $F_{1,106} = 45.99$ ,  $p = 0.000$ ), although this is partly an effect of the treatment. Native algae also occupied most space on vertical ( $F_{1,106} = 17.80$ ,  $p = 0.000$ ) or unshaded plates ( $F_{2,106} = 15.95$ ,  $p = 0.000$ ).

Individual non-indigenous species differed with resident assemblage and orientation, but showed no effect of shading (Fig. 5.6). Several species only occurred on vertical plates including *Diplosoma listerianum*, *Watersipora subtorquata* and *Cryptosula pallasiana* (Fig. 5.6 a - c). *D. listerianum* showed no effect of the other treatments, but *W. subtorquata* differed between sites ( $F_{1,52} = 7.22$ ,  $p = 0.010$ ) and *C. pallasiana* occupied more space on established than new assemblages, although this was only significant at Balmain ( $F_{1,52} = 4.37$ ,  $p = 0.000$ ; SNK  $p < 0.05$ ). Cover of most other NIS (*W. arcuata*, *B. leachi* and *Hydroides elegans*) was greater on vertical than horizontal plates (Fig. 5.6 d - f;  $F_{1,104} = 9.66$ ,  $p = 0.002$ ,  $F_{1,103} = 14.39$ ,  $p = 0.000$  and  $F_{1,106} = 55.55$ ,  $p = 0.000$ ), but often covers were very low so this effect was only detectable at Balmain (SNK  $p < 0.05$ ). The resident assemblage also affected the cover of *H. elegans* ( $F_{1,106} = 27.14$ ,  $p = 0.000$ : New > Established; SNK  $p < 0.05$ ) and *Schizoporella errata* on vertical plates ( $F_{1,104} = 14.71$ ,  $p = 0.000$ : New > Established; SNK  $p < 0.05$ ) (Fig. 5.6 f-g). *Styela plicata* and *Bugula neritina* were the only NIS to show no effect of the treatments, but both occupied < 2% of space (Fig. 5.6 h - i).

Shaded plates received only 8% (Balmain) and 20% (Fig Tree) of the light received by unshaded plates ( $F_{2,330} = 4.51$ ,  $p = 0.012$ ; Fig. 5.7 a). Light levels on the shade control plates were similar to the unshaded plates at Fig Tree although reduced by ~60% at Balmain (SNK  $p < 0.05$ ) suggesting artefacts associated with the use of the Perspex

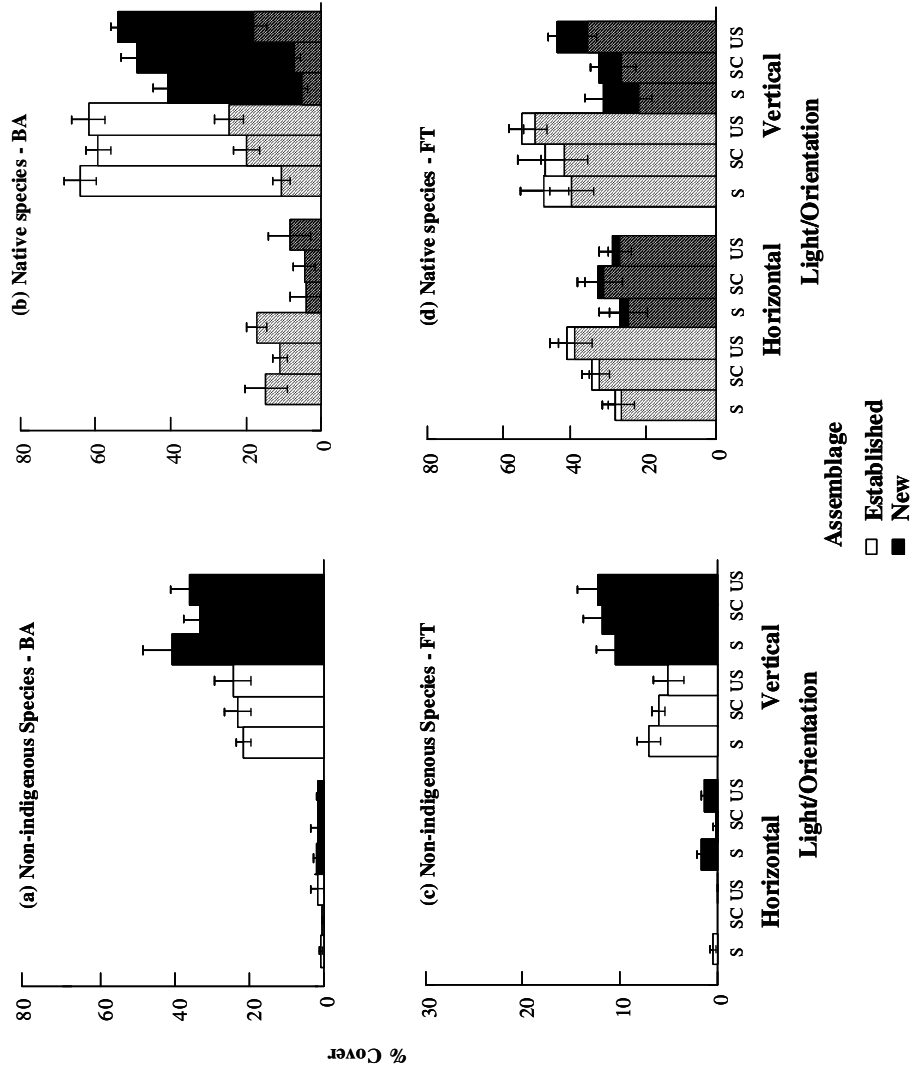


roofs at this location. Lower light levels on shade control plates at Balmain may be related to the higher turbidity ( $F_{1,52} = 36.82$ ,  $p = 0.000$ ) and site sediment loads ( $F_{1,6} = 6.12$ ,  $p = 0.048$ ) at this location (Fig. 5.7 b, c) increasing the sediment loads on perspex roofs. Roofs were cleaned on a weekly basis, but sediment began to accumulate immediately after cleaning at Balmain.

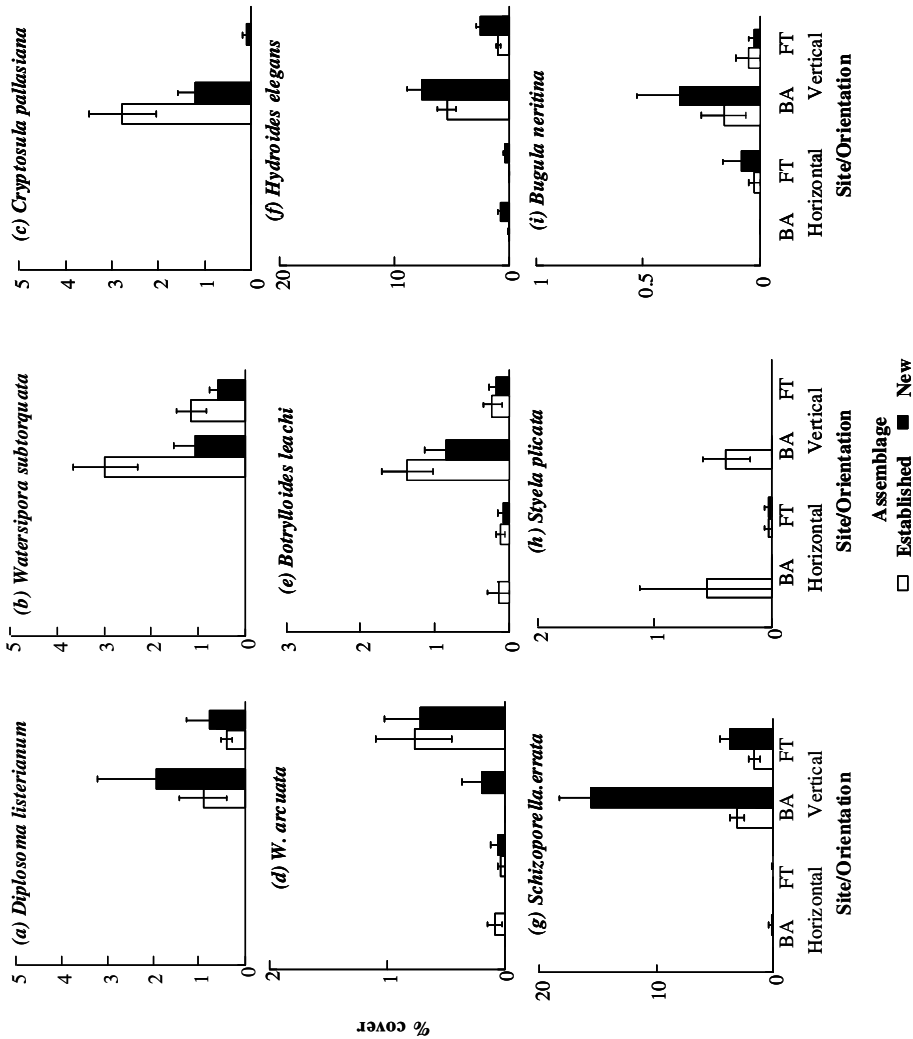
Plate sediment loads differed spatially and were generally greater on horizontal than vertical plates (Fig. 5.7 d, e;  $F_{1,34} = 5.26$ ,  $p = 0.028$ , SNK  $p < 0.05$ ). The presence of Perspex roofs on shaded and shade control treatment reduced sediment on horizontal plates compared to unshaded treatments at one site (Fig Tree – where sediment loads were generally lower and less variable than at Balmain). The roofs were effective at reducing sediment by ~ 31 % (based on the mean sediment collected under shaded, shade control and unshaded treatments). This is comparable to Airolidi and Cinelli (1997) who found that roofs reduced the amount of sediment by 35 %. Vertical plates had negligible levels of sediment, which did not vary between shading treatments. Established and new assemblages did not differ in their sediment loads at either site (Figure 5.7 d, e).

The brown alga *Sargassum* sp. remained the dominant species on the plates and was removed to census the understory community. Dry weight of *Sargassum* sp. varied with resident assemblage, shading and orientation (the latter only at Fig Tree).

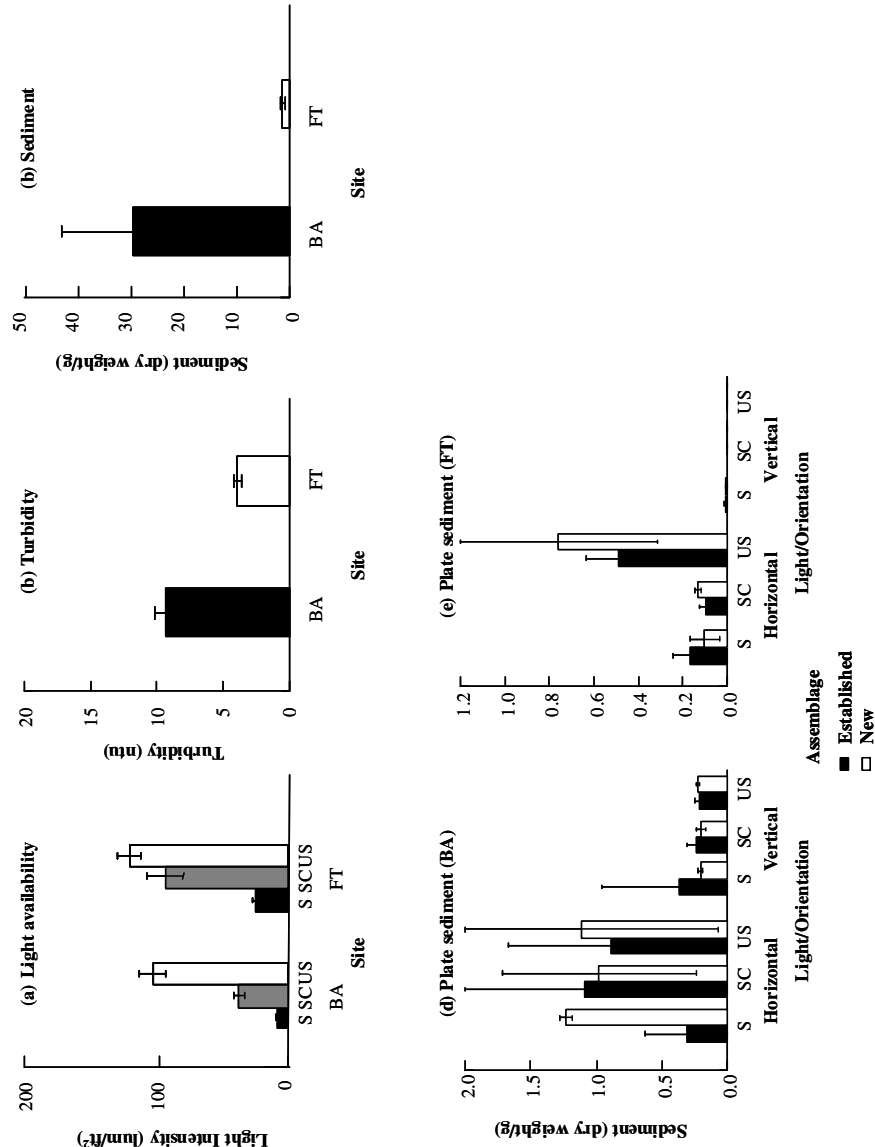
*Sargassum* sp. was most abundant in established assemblages (Balmain -  $F_{1,48} = 11.80$ ,  $p = 0.001$ ) and on horizontal plates (Fig Tree –  $F_{1,48} = 4.07$ ,  $p = 0.000$ ). Shading reduced *Sargassum* sp. abundance (Balmain –  $F_{2,48} = 3.88$ ,  $p = 0.027$ ), particularly on



**Figure 5.5.** Percent cover (+ S.E.) of native and NIS in new and established assemblages on sandstone plates deployed vertically or horizontally at two sites in Port Jackson; Balmain (BA) and Fig Tree (FT). Block colour indicates the proportion that were invertebrates, shading indicates the proportion that were algae. S = shaded, SC = shade control and US = unshaded.



**Figure 5.6.** Percent cover (+ S.E.) of individual NIS in new and established assemblages on sandstone plates deployed vertically or horizontally at two sites in Port Jackson; Balmain (BA) and Fig Tree (FT). Data are pooled for shading treatments as analyses found them to have no effect on percent cover of individual NIS.



**Figure 5.7.** (a) Light intensity (+ S.E.) on sandstone plates at Balmain (BA) and Fig Tree (FT), S = shaded, SC = shade control, US = unshaded. (b)-(c) Environmental conditions (+ S.E.) at BA and FT during the deployment of sandstone plates. (d)-(e) Sediment (mean dry weight/g + S.E.)) on new and established assemblages on sandstone plates deployed vertically or horizontally at two sites in Port Jackson (BA and FT), S = shaded, SC = shade control, US = unshaded.

horizontal and established plates (Fig Tree –  $F_{2,48} = 3.42$ ,  $p = 0.041$  and  $F_{2,48} = 7.89$ ,  $p = 0.001$ ).

## Discussion

Many more non-indigenous taxa were observed on artificial structures compared to the equivalent vertical surfaces of natural rocky reefs located only tens of meters away. However, a restricted set of non-indigenous species were able to recruit to natural rocky reefs and experimental work highlighted the importance of biotic resistance in reducing invasibility. Canopy-forming (on sandstone plates) and turfing (in reef clearings) macroalgae along with high sediment loads provided a barrier to most invaders on horizontal surfaces and, contrary to our hypotheses, patch size and shading had no direct effect on invasion. Reef invaders (primarily sessile invertebrates) appear to mainly threaten vertical rock walls regardless of resident assemblages. NIS richness was two times greater on rock walls (in situ survey) compared to established assemblages on horizontal surfaces (clearings or sandstone plates). The invasive ascidian *Styela plicata* was identified as a particularly successful invader since it was able to recruit to intact assemblages on horizontal surfaces.

### *Non-indigenous species on artificial and natural structures*

The invasibility of a community is closely linked to the utilization of resources by the resident species (Davis et al. 2000). Successful invasion will depend upon the release of resources, coinciding with the arrival of invasive propagules (Clark and Johnston 2005, Britton-Simmons and Abbott 2008) and artificial structures in the marine environment provide ideal conditions for invasion. Resources (space) are made readily available by physical disturbance from cleaning and vessel movements and this

coincides with a continuous supply of invasive propagules (on arriving vessel hulls). Our study found pilings and pontoons to support up to four times more non-indigenous species than on rocky reefs, a greater difference between structures than Glasby et al. (2007). Their comparisons were done at different sites with a combination of in situ and photographic sampling over several time periods and consequently had a lower taxonomic resolution in many instances, while this study was conducted in situ during the same sampling period and repeated twice. These differences might explain the higher numbers of NIS found in the current study.

Anthropogenic modification of estuarine habitats has the potential to reverse competitive interactions between non-indigenous and native species by creating novel environments that make adaptations accrued over evolutionary time redundant (Byers 2002a). Artificial structures provide a novel substrate, floating or fixed and of different composition that some NIS may be better able to exploit than native species. NIS exhibit some preference for shallow floating structures such as pontoons (Lambert and Lambert 1998, Glasby et al. 2007, Dafforn et al. 2009b), potentially because they present a similar surface to a vessel hull with respect to movement and depth. Hull fouling has been recognized as a major source of invaders (Gollasch 2002, Mineur et al. 2007, Pettengill et al. 2007, Piola et al. 2009), and those arriving on vessels will likely have been selected for their preference to settle on shallow floating surfaces. However, this study was unable to detect conclusive differences between NIS diversity on pilings and pontoons. Where individual NIS differed between artificial and natural structures these were often particular to one site or one sampling time. This was potentially due to differences in the substrate composition between sites (Anderson and Underwood 1994, Tyrrell and Byers 2007). The non-indigenous ascidians *Botrylloides violaceus* and

*Botryllus schlosseri* preferentially recruit to artificial substrates (PVC, aluminium, Styrofoam, rubber) (Tyrrell and Byers 2007) and Glasby et al. (2007) found increased occurrence of NIS on concrete and wood substrates compared to sandstone. In the current study, sandstone rocky reefs were dominated by native algal assemblages and only a handful of non-indigenous species had successfully invaded.

*Biotic resistance of turfing and canopy algae*

Native algae are highly adapted to environmental conditions on rocky reefs creating dense, near impenetrable communities that act as a barrier to invading species. Many marine algae possess chemical defences which protect them from epiphytic growth (Steinberg et al. 1997), and turfing algae found in the high subtidal region are particularly resistant to recruitment of other species because the density of fronds creates a cohesive surface layer of sediment (Mangialajo et al. 2008). High light levels on horizontal surfaces promote the growth of turfing algae (Baynes 1999), allowing them to monopolise space despite high sediment loads (Airolidi 1998). *Corallina* sp. can persist for many months when buried beneath sediment (Stewart 1989) whereas many non-indigenous species are invertebrate filter feeders and are smothered by excessive sediment (Ostroumov 2005). We found invertebrate invaders were generally excluded from the established assemblages in the horizontal reef clearings by the resident turfing algae, but took advantage of clearings ('new' assemblages). This suggests that the lack of available space and a resilient native community are providing a barrier to most invaders. However, the algal invaders *Colpomenia sinuosa* and *Dictyota dichotoma* did not differ between established and new assemblages in the horizontal reef clearings. Therefore it could be posited that algal invaders pose the greatest risk to horizontal reef because they do not require bare space and can grow

epiphytically on native algae despite sedimentation. This finding should be treated with some caution as the classification of these algal species is debated and they are often classified as cryptogenic in Australia (Hewitt et al. 2004).

Physical and biological disturbances can create temporary clearings or gaps in algal turf, leaving them vulnerable to invasion. We found that invaders on the reef were largely ephemeral, which suggests propagule supply was patchy and therefore invasion only occurred when our initial disturbance of the clearing coincided with the arrival of non-indigenous propagules (Clark and Johnston 2005, Britton-Simmons and Abbott 2008). Turfing algal communities are particularly well adapted to disturbances and when a gap is created, the surrounding algae will spread vegetatively to quickly reoccupy the space (Airolidi 1998). We hypothesised that smaller clearings would be better able to resist invasion, and found that the encrusting bryozoan *Watersipora arcuata* was largely excluded from our smaller patches (0.05 and 0.10 m<sup>2</sup>). Smaller clearings are rapidly re-colonised by surrounding algal thalli because of the large greater perimeter to area ratio (Airolidi 1998). This reduces the opportunities for colonisation by encrusting species which have little defence against overgrowth by turf (Airolidi 2000). Our inability to detect patch size effects on invasion by the other species could be related to the generally low rate of invasion or the magnitude of difference between patch sizes; the smallest was 0.05 m<sup>2</sup> and largest 0.15 m<sup>2</sup>. Potentially 0.15 m<sup>2</sup> was not a large enough clearing to provide time for an invading species to establish and grow to be resistant to overgrowth. Results may also have been different if we had sufficient space to establish clearings on vertical walls, where invertebrates have been found to dominate (Baynes 1999).



Conditions for invading species differ greatly between turfing and canopy-forming algal communities. Canopy-forming algae can act as habitat modifiers by casting shade over the understory and altering flow regimes (Kennelly 1983, 1989, Schmidt and Scheibling 2007). Reduced light and restricted flow under the canopy has the potential to affect the supply of larvae and algal propagules and can inhibit the development of benthic assemblages (Kennelly 1989). The fronds of canopy algae can also scour the substrate, removing any invertebrate recruits that may have settled (Connell 2003). The native alga *Sargassum* sp. forms a dense canopy over rocky reefs and in the current study was the primary space occupier of the sandstone plates. We found that percent cover of native algae decreased with reductions in light shaded plates and also showed some negative effects of sediment. Canopy-forming algae such as *Sargassum* sp. are less resistant to sedimentation than turfing algae (Airolidi 2003) and the high levels of sedimentation on horizontal plates may have been sufficient to reduce their cover. We also found evidence that non-indigenous invertebrates were able to invade the understory community. This contrasts with results from previous experiments that manipulated canopy algae and invertebrates (Connell 2003) potentially due to the morphology of the species involved in each study, or the availability of non-indigenous propagules that may be better able to tolerate conditions in the understory community than native invertebrates.

#### *Identifying management priorities on the reef*

Many sessile invertebrate larvae exhibit behavioural adaptations to avoid settlement near a competitive dominant, including negative photo-taxis which results in them settling in shaded areas (vertical surfaces of pilings, pontoons and on rock walls and under overhangs) (Thorson 1964, Young and Chia 1984). Previous studies have found

orientation and shading to interact in the colonisation of natural substrates with shading increasing the cover of invertebrates such as serpulid polychaetes, bryozoans (including *Watersipora subtorquata*) and ascidians (including *Styela plicata*) primarily due to a reduction in algal cover (Glasby 1999c, b, 2000, Miller and Etter 2008). In the current study, the encrusting bryozoans *Watersipora subtorquata* and *Schizoporella errata* were much more abundant on the vertical sides of pilings and pontoons than rocky reefs and also occupied most space on vertical sandstone plates (similar to Glasby 1999a). These data are consistent with both competitive exclusion by algae and extremely high sediment loads on horizontal plates (despite the presence of shades) acting as a deterrent to settlement.

Competitive interactions play a major role in determining the composition of hard substrate assemblages (Jackson 1977). The encrusting bryozoan *Schizoporella errata* is known to exclude other species from the space it occupies, however its larvae rely on bare space for successful colonisation (Sutherland 1978). We found that *S. errata* was significantly more successful at invading new assemblages (i.e. bare sandstone plates) and occupied between 3 – 15 times more space than other invaders over the 8 months of the study. Serpulids such as *H. elegans* are also thought to be good colonisers, but poor competitors for space (Jackson 1977, Johnston and Keough 2003), a likely factor in its invasion of the new assemblages in horizontal reef clearings. The physical disturbance from high sedimentation loads may have also advantaged the serpulid by keeping recruitment of more competitive (but less sediment tolerant) organisms such as the ascidians or bryozoans low (Johnston and Keough 2003). These invaders could pose a threat to disturbed patches on rock walls where primary substrate is available and sediment loads are reduced. Invasion by competitive-dominant NIS such as *S. errata* is

likely to prevent recovery by the exclusion of subsequent settlers (Sutherland 1978). While most NIS invaded new assemblages, several (the encrusting bryozoans *Watersipora subtorquata*, *Cryptosula pallasiana* and the colonial ascidian *Botrylloides leachi*) recruited most to established assemblages on vertical plates, suggesting that when sedimentation levels are lower, and light levels are reduced, resident assemblages are more vulnerable to invasion.

Much research has focused on the facilitative interactions between invasive species (Simberloff and Holle 1999, Ricciardi 2001, O'Dowd et al. 2003, Floerl et al. 2004, Grosholz 2005), but comparatively less well known are the effects of facilitative interactions between native and non-indigenous species. Traditionally native species are thought to act as a barrier to invading species by occupying available ecological niches and utilisation of resources (Tilman 1997, Stachowicz et al. 2002a, Stachowicz and Byrnes 2006, Fridley et al. 2007). The presence of native predators or parasites that might confer resistance if they preferentially prey on or parasitise invading species (Byers 2002a, Bishop et al. 2006). Similarly, novel attributes of an invading species might confer protection from a predator or parasite (Colautti et al. 2004). Potentially the NIS identified in the current study are spatially excluded from the reef by algae and where space is available they are subject to greater predation pressure through lack of a refuge. However in some instances native species might create conditions that actually promote the establishment of NIS. The solitary ascidian *Styela plicata* is identified as a pest species in Australia with ecological impacts because it competes with native species for resources (Hayes et al. 2005). *S. plicata* was the only non-indigenous invertebrate that invaded established assemblages in horizontal reef clearings and sandstone plates. It is not clear why *S. plicata* succeeds where other invaders do not,

but one likely explanation pertains to the ascidian's use of arborescent species as refugia during its vulnerable juvenile stage (Sutherland 1974, Mook 1983). Fish predation is an important source of mortality for young *S. plicata* and the ascidian dominates space when fish are excluded or if arborescent species (such as hydroids and bryozoans) are present (Mook 1983). The reef clearings were primarily occupied by turfing algae that may have created a similar refuge for settling *S. plicata* larvae. Tight packing of fronds in algal turf reduces grazing pressure (Hay 1981) and other mobile invertebrates are thought to shelter from fish predation beneath the turf (Grahame and Hanna 1989). Native algae may in fact be facilitating the establishment of *S. plicata* and further investigation of this theory is necessary to test its validity. Invasion by *S. plicata* poses a double threat to the reef as the ascidian provides a suitable substrate for settlement of other NIS (Fig. 5.8) and if dislodged has the potential to free up relatively large amounts of space that other NIS can recruit to (Sutherland 1978).

## Conclusion

Community ecology of natural rocky reefs suggests that native algae dominate well-lit horizontal surfaces while native sessile invertebrates are confined to shaded vertical surfaces. The combination of anthropogenic modification and increased invasive inoculation in estuaries is threatening this balance. The construction of artificial substrates provides additional novel substrate for colonisation by invasive propagules and reduces distances between hard substrates, which has the potential to deliver more propagules to the rocky reef habitats. Light facilitates native algae dominance on horizontal reef surfaces, but where gaps are created either from physical (e.g. anchoring) or biological (e.g. grazing) disturbances, opportunistic NIS may be able to invade. In highly turbid estuarine environments, sediment plays an important role in

preventing the persistence of NIS on horizontal reef by smothering new recruits or clogging filter feeding apparatus. Some NIS (including the solitary ascidian *Styela plicata*) are able to grow epiphytically on native algae, which may increase their chance of escape from sediment cover and aid in their successful establishment on horizontal rocky reefs. Further investigation of the relations between NIS and native algae on the reef are necessary to identify positive interactions that may facilitate future invasions.



**Figure 5.8.** The solitary ascidian *Styela plicata* (cream) facilitates recruitment of the encrusting bryozoan *Watersipora subtorquata* (red) to the reef.

## Chapter 6

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### Summary.

The aim of this thesis was to examine anthropogenic disturbances in estuaries and their effects on the establishment and distribution of marine invaders. Specifically I investigated the role of antifouling paints, artificial structures and physical disturbance in the invasion of estuaries. There was no difference in the distribution of non-indigenous species between commercial and recreational harbours. However, the distribution of several non-indigenous and exported species (collectively termed invaders) were positively correlated with increasing pollutant loads (copper and in some cases tributyltin). Physico-chemical variables including pH, temperature and turbidity were also important predictors of the distribution of fouling species and for three organisms, they explained > 50% of the variation in percentage cover across four estuaries. Disturbances that alter environmental conditions therefore have the potential to shift both native and non-indigenous species distributions.

The construction of artificial structures in the marine environment might also affect species distributions by providing an entry point for marine invaders. Non-indigenous species (NIS) were more diverse on artificial structures than natural rocky reef and invaders were most abundant on shallow floating structures, which present similar conditions to a pontoon or vessel hull. Few NIS were present on natural rocky reefs and both sedimentation and the presence of a native algal assemblage were found to enhance resistance to invasion in this habitat. Vertical sandstone surfaces were most heavily invaded regardless of the availability of bare space. My findings suggest that invasion opportunities will be greatest for non-indigenous invertebrates on vertical rock walls and that some NIS will benefit from disturbances that create clearings on the reef.

## Management implications

Large-scale observational surveys are important for the identification of ecological patterns (Underwood et al. 2000). My thesis relied on the identification of ecological patterns in the field and subsequent manipulative experiments under field conditions to distinguish the contributions of several factors to marine invasion. My research has demonstrated important relationships between anthropogenic disturbances and the distribution of NIS in estuaries.

### *Antifouling paints as agents of disturbance*

I used manipulative experiments to show that heavy metals from antifouling (AF) paints may promote the establishment of some invaders, a similar result to Piola and Johnston (2008a). However, I tested a range of antifouling paints, including copper (conventional and SPC) and tributyltin, and used the results to explain species distributions in relation to water quality data collected from four estuaries. Importantly, I found that heavy metal contamination and physico-chemical variables were more important correlates of species distributions (including ten invaders and one native species) than the dominant estuary type (recreational vs. commercial). This has implications for the management of vectors as well as ports and marinas which may act as sources of propagules for invasive species.

To test whether levels of heavy metals in the water column differed between commercial and recreational estuaries, the accumulation of metals was measured in experimentally deployed oysters. I found tributyltin contamination to still be a significant problem (at levels that could cause oyster deformities) in recreational estuaries despite the presence of a ban on the application of TBT AF paints (since 1989)



and other studies suggesting widespread recovery from the ecological impacts of TBT (e.g. reduced incidence of imposex Evans et al. 1995). It is possible that some recreational boats continue to illegally use TBT, but these results are more likely to result from the variable persistence of TBT in the environment. We know that TBT is rapidly adsorbed onto particulate material (Clark et al. 1988) and the accumulation of sediments can provide a source of future TBT contamination in the water column if these particles are re-suspended (OC 2005). However we do not know whether some ecosystems will recover without assistance so remediation may be necessary in the areas identified as hotspots for TBT contamination e.g. marinas and port areas (Gibson and Wilson 2003). The prohibition of tributyltin (TBT) in AF paints is a relatively recent event (IMO 2001) and as such my work presents a baseline for monitoring the recovery of heavily contaminated estuaries.

Heavy metal contamination is a primary concern in many estuaries because of the toxic effects on marine communities and has been the subject of numerous studies [e.g. (Champ and Seligman 1996, Hall Jr et al. 1998)]. Copper is found naturally in the marine environment and at low levels is an important element in organism metabolism (Lewis and Cave 1982). At higher concentrations, copper becomes toxic to many marine species and also has many sublethal effects including reduced growth and fecundity (Lewis and Cave 1982). My research identified several marine invaders that are highly tolerant to copper and show increased spatial dominance in areas where copper has accumulated e.g. sheltered port areas and marinas. This agrees with findings by Piola and Johnston (2008a) who were able to show that dosing fouling communities with copper increased invader dominance at the expense of native species. My research has added to a growing body of literature linking copper to marine invasion (Allen

1953, Floerl et al. 2004, Piola and Johnston 2008a) by providing evidence that the use of copper AF paints may be driving the export of Australian native species to other locations. Evidence of copper tolerance in an endemic species may be a useful predictive tool to identify future candidates for export. My results suggest that steps should be taken to reduce the contaminant loads in estuaries by gradually phasing out the use of copper in AF paints and funding research into effective non-toxic alternatives. Interim options include regulation of copper release rates from AF paints and regulations controlling the disposal of copper waste from shipyards.

#### *Artificial structures as agents of disturbance*

This research also has important implications for the management of artificial structures in estuarine systems. I conducted in situ surveys of artificial structures and natural reef and found a greater difference in NIS richness between structures than Glasby et al (2007), potentially related to the taxonomic resolution of our sampling techniques. I found NIS were up to 4 times more diverse on artificial than natural structures. I was also able to show experimentally that movement and depth affect recruitment of marine invaders. Specifically that shallow moving structures promote invader dominance, potentially because they present a similar structure to the vessel hulls which are important vectors for the transport of NIS. My findings suggest that invasion levels might be reduced if pilings and pontoons were constructed to be more similar to natural reefs. Past research has shown that modifying the materials used in construction so that they have a similar texture and surface complexity to sandstone would go some way to removing opportunities for NIS allowing the establishment of native assemblages instead (Glasby et al. 2007, Moreira et al. 2007). My results suggest that the resident algal assemblage is acting as a barrier to invasion on reefs by competitively excluding

recruiting NIS. I found strong evidence to support this hypothesis with NIS largely excluded from areas where algae were present. Further tests of this hypothesis could include seeding artificial structures with native algal transplants and providing an artificial light source under the structure. Predation levels on artificial structures and natural reef should also be considered as another possible exclusion mechanism (Reusch 1998).

#### *Invasibility of natural reef*

While the native algal assemblages sampled during my research appear to be highly resistant to invasion, a restricted set of NIS were found on the reef suggesting the potential for them to establish in natural communities. My results suggest that macroalgae provide the main barrier to invasion on rocky reefs, and factors such as disturbances that reduce algal abundance may create opportunities for NIS (Levin et al. 2002). Examples of physical disturbances in estuaries observed during my study included vessel anchoring, which dragged an experimental frame and has the potential to remove large algal patches, and swash from passing vessels that dislodged and moved small boulders (pers. obs.). These vessel disturbances could be managed in a similar way to seagrass habitats by introducing regulations regarding anchoring and vessel speeds (Lloret et al. 2008).

My results suggest that the other main barrier to invasion on horizontal rocky reefs is sedimentation. High sediment loads are likely to increase invasion resistance if conditions result in the smothering of recruits or sediment clogs the filter-feeding apparatus of invertebrate invaders (Ostroumov 2005). Macroalgae are highly tolerant of sedimentation, suggesting that this information could be integrated into a management

plan to eliminate invaders on the reef. However, the controlled dumping of sediment to inhibit invertebrate invaders would be risky without an understanding of appropriate levels of sediment and potential non-target effects e.g. native invertebrates (mobile and sessile). Instead of responding to the threat once invaders have established on the reef, my research suggests that it might be possible to target them at the propagule source, which appears to be artificial structures. Past concerns surrounding invader establishment on artificial structures have resulted in eradication efforts [e.g. *Didemnum vexillum* in New Zealand (Coutts and Forrest 2007)]. These include wrapping and chlorine treatment of floating structures and wrapping of pilings. Such treatments can be effective at removing the invader from the particular structure, providing there are no other propagules sources in the area (Coutts and Forrest 2007).

In Australia there currently exists a system that co-ordinates monitoring and response to marine invasions (National System for the Prevention and Management of Marine Pest Incursions). I monitored reef assemblages for 32 months and found that few NIS are able to invade horizontal rocky reef, however some NIS may pose a threat to rock wall communities and behave opportunistically when bare space is created on horizontal reef. Regular monitoring by the National System should target rocky reef assemblages to assess the status of known invaders and to detect any new invaders. Currently, it appears the NIS present on horizontal reefs exist ephemerally and in low numbers, therefore they are unlikely to have major impacts on the native assemblages. However my survey work found NIS richness in established assemblages on vertical reef walls was two times greater compared to established assemblages on horizontal surfaces (reef clearings and sandstone plates) suggesting greater potential for impact on reef walls

than reef flats. Continued monitoring is necessary to assess the present level of impact and to detect future threats.

### **Future directions**

The invasibility of a system has been linked to the presence of native predators or parasites that might confer resistance if they preferentially prey on or parasitise invading species (Byers 2002b, Bishop et al. 2006). Similarly, novel attributes of an invading species might confer protection from a predator or parasite (Colautti et al. 2004). I found that the solitary ascidian *Styela plicata* was able to invade established assemblages on horizontal reefs and hypothesised that this might be due to native algae providing a refuge. Invasion by *S. plicata* poses a double threat to the reef as the ascidian provides a suitable substrate for settlement of other NIS and if dislodged also has the potential to free up relatively large amounts of space that other NIS can recruit to (Sutherland 1978). The ascidian is vulnerable to predation during its early juvenile stages (Mook 1983) and the complexity of turfing algal fronds may provide a level of protection from grazers. I did some preliminary experiments manipulating the propagule supply of this ascidian on the reef, along with the resident assemblage in grazer exclusion plots, but recruitment was unsuccessful. Potentially the grazers present on the reef are providing an added barrier to some of the invaders identified in this study and this should be tested in further experiments on the reef.

Conditions for invading species are likely to differ between turf and canopy algae. Turfing algae are particularly resistant to recruitment of other species because the density of fronds creates a cohesive surface layer of sediment (Mangialajo et al. 2008), while canopy-forming algae have the potential to alter flow regimes and reduce the

supply of invasive propagules to the developing benthic assemblage underneath the canopy (Kennelly 1989). In addition, physical abrasion by canopy algae sweeping the substrate can reduce recruitment to the understory (Connell 2003). My research considered the invasibility of turf and canopy macroalgae and found both to be highly resistant to invasion by most NIS, although the mechanism of resistance is not fully understood. Further experimental work is needed to compare the morphology between turf and canopy algae, e.g. the density and arrangement of fronds to identify particular characteristics that might prevent invasion.

In addition, many algal species are known to produce secondary metabolites that reduce epiphytic growth (Steinberg et al. 1998), and so could potentially inhibit recruitment of an invading species. Of particular interest is whether the secondary metabolites produced by native algae are equally effective at preventing the recruitment of native and non-indigenous species. This could be tested by obtaining larvae from multiple species and running mesocosm experiments in the field or laboratory. While secondary metabolites are generally produced to inhibit the recruitment of fouling species, the presence of biofilms on submerged surfaces can provide cues for the settlement of some species and actually increase adhesion strength (Zardus et al. 2008). This thesis compared macro-organisms fouling artificial and natural structures, but another consideration would be whether the communities of micro-organisms colonising artificial structures are similar to those on natural reef, and what role different micro-organisms play in the recruitment of sessile invaders.

## **Final Remarks**

This thesis was undertaken with the aim of examining the effect of shipping-related disturbances, including pollution and the construction of artificial structures, on the invasibility of estuarine systems. A combination of field surveys and manipulative experiments were successfully employed at multiple sites to address this aim. Surveys of settlement plates and quadrats (in situ) were used to identify non-indigenous species distribution patterns in relation to estuarine pollution and between artificial and natural structures respectively. Invaders (non-indigenous and exported species) were sensitive to the presence of pollution and dominated artificial structures. I was able to show experimentally that the presence of copper and in some cases tributyltin can enhance recruitment of different invaders and also aid in identifying species that may be future candidates for export. Through manipulative field experiments I was able to show that shallow floating structures (analogous to pontoons or vessel hulls) promote invader dominance, while few non-indigenous species are able to invade natural rocky reefs. Finally, there is some evidence of an invasion threat to natural reefs, particularly vertical rock walls and when disturbances create space (e.g. anchoring or swash). Impact studies are necessary to further monitor and prioritise the management of particular invasive species.

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**Appendix I:** Dafforn, K. A., T. M. Glasby, and E. L. Johnston. 2009. Links between estuarine condition and spatial distributions of marine invaders. *Diversity and Distributions* **15**:807-821.





## Links between estuarine condition and spatial distributions of marine invaders

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### ABSTRACT

**Aim** Non-indigenous species pose a significant threat to the environment and to global economies. Predictive and preventative measures are widely considered more effective in curtailing invasions than are eradication or control measures. Of key importance in the prediction of regional invasion risk are the environmental conditions that enable successful establishment.

**Location** We surveyed native and non-indigenous sessile invertebrate diversity in each of two commercial (600–1500 vessels per year) and two recreational estuaries (seven to nine marinas) in New South Wales, Australia.

**Methods** A nested hierarchical design was employed to investigate variation in sessile invertebrate diversity at the scales of site (1–3 km apart) and estuary (40–180 km apart). Settlement plates (15 × 15 cm) were used to sample invertebrates and background heavy metal loads were assessed using bioaccumulation in experimentally deployed oysters. Other physico-chemical variables were monitored monthly. Manipulative experiments were used to test the direct effects of exposure to copper and tributyltin (TBT) antifouling paints on sessile invertebrates.

**Results** Native and non-indigenous species richness differed at various spatial scales, but showed no consistent difference between commercial and recreational estuaries. Instead, individual species distributions were strongly related to metal contamination, temperature, turbidity and pH. In experimental studies, several species (mostly invaders) were more abundant on plates exposed to copper and/or TBT antifouling paints. We found higher levels of copper (and in some instances TBT) in recreational marinas than in commercial harbours.

**Main conclusions** Our results demonstrate the importance of metal pollution and physico-chemical variables in the establishment of invaders in new regions. We have identified several native Australian species that have been exported overseas and suggested mechanisms contributing to their transport and establishment. Combining physico-chemical information about donor and recipient regions with species tolerances could go some way to predicting where future invasions may occur.

### Keywords

Biological invasions, copper, fouling assemblage, recreational vessel, shipping, tributyltin.

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### INTRODUCTION

The introduction of non-indigenous species (NIS) is widely argued to be the second most important cause of native species decline after habitat loss (Vitousek *et al.*, 1996). NIS have been

associated with native species extinctions through predation, competition and habitat alteration (Mack *et al.*, 2000). The reduction in native species abundances has associated economic costs, particularly when the species at risk is one of interest to farming or aquaculture. In the United States alone,

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damages and losses from NIS amount to \$138 billion per year (see reviews by Pimentel *et al.*, 2000; Colautti *et al.*, 2006). Recognition of the threat NIS pose to the environment and global economies has resulted in a push for predictive and preventative methods to control the spread of invaders (Mack *et al.*, 2000; Hulme, 2006; Keller *et al.*, 2008). To effectively manage the invasion threat, managers need to be able to identify vulnerable areas where NIS will dominate (e.g. 'invasion hotspots' Holeck *et al.*, 2004) and where they may act as a source of propagules to neighbouring regions.

Of key importance in the assessment of regional invasion risks are the environmental conditions that allow for establishment and dominance of species in a new region. The so-called 'habitat suitability' hypothesis suggests that successful introduction is more likely if species are matched with suitable environments (Williamson *et al.*, 1986; Blackburn & Duncan, 2001). The invasion process is likely to select for species that have a wide environmental tolerance because they must survive the process of entrainment and transport (Johnston *et al.*, 2009). In the marine environment, ports and marinas are recognized as invasion hotspots (Carlton, 1987) and transport vectors include the hulls of boats, ballast tanks or sea chests (Allen, 1953; Williamson *et al.*, 1986; Ruiz *et al.*, 2000; Coutts *et al.*, 2003). Conditions for invaders transported internally (ballast water) and externally (hull fouling) by maritime vessels can be highly stressful (Wonham *et al.*, 2001; Minchin & Gollasch, 2003). In addition to surviving high concentrations of heavy metals on the hulls and in the ballast tanks of vessels (Murphy *et al.*, 2002; Finnie, 2006; Hua & Liub, 2007), marine invaders may be exposed to physical hardships during an oceanic voyage caused by the ship's movement through the water and dramatic changes in the physico-chemical properties of the water body between geographical regions. Disturbances such as changes to temperature regimes and pollution in the recipient environment can also increase the susceptibility of a community to arriving invaders (Stachowicz *et al.*, 2002; Clark & Johnston, 2005).

Ports and harbours are ideal systems within which to test theories about associations between invaders and environmental parameters. They are the first point of entry for NIS arriving on the hulls of vessels, in ballast tanks or sea chests (Coutts *et al.*, 2003), although ballast water discharge from international vessels is now prohibited in Australian ports if the water is foreign, marine, coastal or unexchanged (AQIS 2008). Ports are also important foci of anthropogenic activities that can influence physico-chemical variables such as temperature, salinity, dissolved oxygen (DO), pH and turbidity. Changes in these variables may affect the growth and reproduction rates of resident species and alter their metabolic rates and feeding efficiencies (Salazar & Salazar, 1996; Ostroumov, 2005). Physico-chemical variables also have been found to influence the distribution of many marine species, (Barry *et al.*, 1995; Engle & Summers, 1999; Akin *et al.*, 2003; Roessig *et al.*, 2004; Nicholson *et al.*, 2008), and some studies have focused particularly on NIS. For example, Miller *et al.* (2007) found that the ability to withstand low salinity was an important

predictor of molluscan invader success. Similarly, in the brackish waters of Europe, NIS were found to exploit environmental conditions that did not favour native species (Paavola *et al.*, 2005). Levels of DO have also been found to affect interactions between native and non-indigenous species (Byers, 2000; Jewett *et al.*, 2005).

Heavy metal contaminants in estuaries can also influence the distribution of marine species and have been well studied in benthic communities (Brown *et al.*, 2000; Morrissey *et al.*, 2003), but comparatively little remains known about their effect on sessile invertebrate fouling communities (marine organisms that grow on hard substrates). The build-up of heavy metals in estuaries has been posited as a major driver of invasion outcomes through the provision of a competitive advantage to more tolerant NIS (Piola & Johnston, 2007). Although there is experimental evidence to support this notion (Piola & Johnston, 2007), there are no data directly linking pollution levels and invasive marine species' distributions in the field. Levels of pollution will differ with respect to the primary activities in an estuary and will differ spatially and temporally within an estuary. For example, estuaries dominated by industry are likely to have high levels of contaminants from factory waste (particularly metals) and may accumulate high concentrations of tributyltin (TBT) from antifouling (AF) paints used on commercial vessels (e.g. oil tankers and coal transporters) (Lewis, 2001; Lewis *et al.*, 2004). Estuaries surrounded by residential housing and occupied by recreational marinas are likely to have lower levels of heavy metals, although copper from the AF paints used on recreational vessels has been found to accumulate in the water around marinas (Claisse & Alzieu, 1993; Floerl & Inglis, 2003; Schiff *et al.*, 2004; Warnken *et al.*, 2004).

Antifouling paints are applied to the external submerged surface of maritime vessels and often contain heavy metals to prevent the settlement of fouling species. Since the 1980s, most recreational vessels (< 25 m long) in developed countries have been banned from using TBT-based AF paints (Champ, 2000). These recreational vessels have generally reverted to traditional copper-based AF paints, while commercial vessels continued to use TBT [although use of TBT has been gradually phased out since 2003 and banned since January 2008 by countries ratifying the IMO convention (IMO 2001)]. Based on the predicted half-life of TBT and associated compounds, many recreational estuaries should now harbour only very low levels of this contaminant in the water column (Champ & Seligman, 1996).

This study examined the prediction that commercial and recreational estuaries support distinct populations of non-indigenous and native with more NIS in commercial ports. It is proposed that these patterns of fouling species' distribution are related to different numbers and types of transport vectors in the two types of estuaries and to the different contaminants and physico-chemical conditions present in each. We also examined the response of non-indigenous and native fouling species to two copper-based and one TBT-based AF paint to test the hypothesis that the presence of copper AF paints would

favour marine invaders. Results are interpreted with reference to species that are non-indigenous to Australia and species that are native to Australia, but have been introduced overseas; the latter are termed 'exported' species (ES) (Dafforn *et al.*, 2009). Hereafter, NIS and ES are referred to as 'invaders'. It is important to consider invaders as a specific group including not only NIS, but also native species that pose a risk of export to other regions.

## METHODS

### Study sites

Experiments were conducted in two commercial and two recreational estuaries along the coast of New South Wales, Australia, between June 2006 and February 2007 (Fig. 1). Port Kembla and Newcastle Harbour are commercial estuaries receiving international vessel traffic. Port Kembla receives c. 600 vessels per year (P.K.P.C., 2006) and Newcastle Harbour 1500 vessels per year (N.P.C., 2006). Both harbours have a single small recreational marina located > 500 m away from the main port area, but each harbour is dominated by commercial facilities. Pittwater and Port Hacking are exclusively recreational estuaries and neither

supports commercial shipping activities. Pittwater has nine marinas and two sailing clubs and Port Hacking has seven marinas and hundreds of private pontoons and moorings accommodating an estimated 50–200 boats at each marina (Dafforn, pers. obs.). A spatially nested hierarchical design was employed to investigate variation in species recruitment within these estuaries. Three sites (c. 1–3 km apart) were sampled in each estuary. Each recreational site was situated at a marina, and each commercial site was situated at a docking terminal (Fig. 1).

### Biodiversity survey and experimental comparison of antifouling paints

To sample sessile invertebrate (fouling) assemblages in these estuaries, six settlement plates (controls) (15 × 15 cm) were deployed at each site at a depth of 2 m below mean low water springs (MLSW) (the estuaries have tidal ranges of between 1 and 2 m). Settlement plates were made of black Perspex (3.5-mm thick) and were roughly sanded. Control plates were attached to plastic frames using two cable ties in the outer 3 cm. Frames were suspended vertically and were hung in shaded places, either under pontoons or on pilings beneath jetties c. 2–3 m from the sea floor at MLSW. To test hypotheses about effects of AF paints on sessile invertebrate assemblages, an additional 18 settlement plates were treated with one of three different paints and simultaneously deployed at each site; six were treated with a 3-cm border of 'Micron Extra' (copper diuron, CuDi), six with 'Intersmooth 360' (copper zinc pyrithione, CuZnP) and six with 'Superyacht 800' (TBT). CuDi is commonly used by recreational vessels, while CuZnP is only available to commercial vessel owners and has been introduced as an alternative to TBT. Control plates (described above) were treated with a 3-cm border of non-toxic primer to allow direct comparisons with the paint treatments. Settlement plates were deployed on three frames with two replicates of each AF treatment on each frame (eight plates per frame). Frames were separated by 3–5 m within each site.

Jetties and pontoons are the dominant structures in commercial and recreational estuaries and therefore, for logistic reasons and to reflect the primary substrate available in those areas, it was necessary to attach frames to stationary structures (jetties) in commercial estuaries and to moving structures (pontoons) in recreational estuaries. Research by Holloway & Connell (2002) found differences in assemblage development between stationary and moving structures; however, they compared moving plates that were either partially above the surface or submerged on the water line, and did not test effects at greater depths. To investigate whether such structural differences may have confounded our comparison of assemblages between estuary types, we deployed 11 × 11 cm Perspex settlement plates attached to larger frames that were moving or stationary at 0.5 or 2 m depth for a period of 3 months (Dafforn *et al.*, 2009). Invaders (NIS + ES) were more numerous on moving than stationary structures at

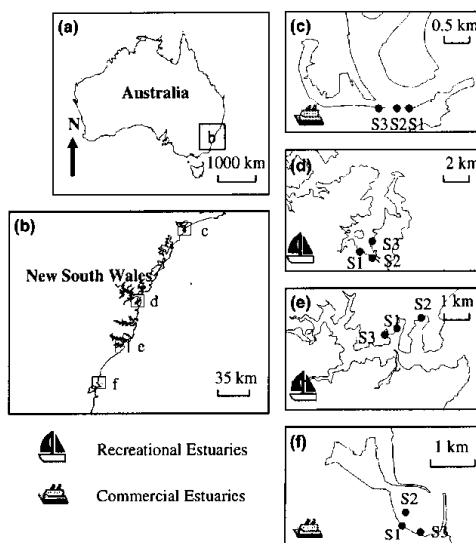


Figure 1 Sampling locations of berths and marinas. (c) Newcastle Harbour containing S1 = Dyke 1, S2 = Dyke 2 East and S3 = Dyke 2 West, (d) Pittwater containing S1 = The Quays Marina, S2 = RPAYC and S3 = Heron Cove Marina, (e) Port Hacking containing S1 = Burraneer Bay Marina, S2 = Cronulla Marina and S3 = Dolans Bay Marina and (f) Port Kembla containing S1 = Jetty 4 South, S2 = Jetty 4 North and S3 = Jetty 6.

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0.5 m; however, they showed no difference between moving and stationary structures at 2 m (Dafforn et al., 2009), which was the depth at which plates were deployed in the present study. Similarly, when species were analysed individually, there was no difference between stationary or moving structures at 2 m for several of the species that were dominant in this study (Dafforn et al., 2009). In addition, patterns for other dominant species were generally in the opposite direction to those found between commercial and recreational estuaries. For example, some invaders have been found to be more abundant on moving plates at depths of 2–3 m, including *Diplosoma listerianum*, *Pyura stolonifera*, *Amphibalanus variegatus* and *Bugula neritina* (Glasby, 2001; Dafforn et al., 2009). In the present study, these species occupied more space in commercial estuaries, where the frames were always attached to stationary structures. As such, our comparison of communities between commercial and recreational estuaries may have failed to detect differences between estuary types, for some species, because the sampling in recreational estuaries was biased towards some invaders and as such potentially overestimated their abundance in recreational estuaries.

#### Data collection and analysis

After 8 months, settlement plates were retrieved, photographed and preserved in 5% formaldehyde. Images were used to estimate percentage covers of taxa using one hundred randomly arranged points placed over the central  $9 \times 9$  cm area. Organisms were identified to species level where possible and identities were confirmed by examination of preserved plates. Species that could be identified were classified as native, non-indigenous or exported. Those that could not be identified or whose origins were uncertain were classified as cryptogenic (Carlton, 1996a).

Data for total non-indigenous, native (including exported) and cryptogenic species richness (number of species) and percentage cover on control plates were compared between estuary types using a three-factor nested analysis of variance (ANOVA). 'Estuary Type' was treated as a fixed orthogonal factor and 'Estuaries (within Estuary Type)' and 'Sites (within Estuaries)' were random, nested factors. All data were assessed for normality using residual frequency histograms and for homogeneity of variance using Cochran's C-test. We then tested for a correlation between non-indigenous and native species richness and results are presented graphically. Percentage covers of dominant species (> 5%) on control plates were also compared between estuary types using a three-factor nested ANOVA (as described above).

To investigate the effects of AF paints, we compared results of species' patterns across all experimental units to test whether the patterns of difference among control and AF treatments occurred more frequently than would be expected by chance (Binomial test, Underwood, 1997). AF treatments were analysed separately for each estuary, and frames were the replicates in these analyses ( $n = 18$  in each estuary type).

#### Comparison of heavy metals and physico-chemical variables between estuaries

To test whether levels of heavy metals differed between commercial and recreational estuaries, the accumulation of metals was measured in experimentally deployed oysters. Oysters (and settlement plates) were spaced appropriately to prevent cross-contamination from the different AF paints. Dafforn et al. (2008) took water samples from directly next to settlement plates in an attempt to measure the release of copper and TBT from the painted borders and found that levels were below detectable limits ( $< 5 \mu\text{g L}^{-1}$  copper and  $< 2 \text{ ng L}^{-1}$  TBT). Three mesh bags of 10 oysters were suspended at 2 m depth at each field site for 12 weeks, and then collected and depurated for 48 h in containers of filtered sea water before storage at  $-10^\circ\text{C}$  (Robinson et al., 2005). Each replicate ( $n = 3$  per site) consisted of a composite of four oysters selected randomly from a single mesh bag, freeze dried and ground to powder following Hardiman & Pearson (1995). Subsamples of 0.4 g of freeze-dried oyster powder were added to 5 mL of distilled  $\text{HNO}_3$ , 2 mL of  $\text{H}_2\text{O}_2$  and 3 mL of Milli-Q water in digestion vessels and microwave digested at  $190^\circ\text{C}$  for 20 min. After digestion, samples were made up to 30 mL using Milli-Q water and analysed using ICP-MS at the Solid State and Elemental Analysis Unit (UNSW, Sydney). Each sample was analysed for Al, As, Cd, Co, Cu, Hg, Ni, Mn, Pb, Sn and Zn. Recoveries were generally within 90–100% of expected values (NIST 1566b Oyster Tissue). Where recoveries were outside this range, the data were omitted from analysis (Al, Hg, Ni, Pb and Sn). Because of costs of TBT analysis, three replicates were analysed per estuary (one per site) by the National Measurement Institute (Sydney).

To test for differences in physico-chemical variables between sites, temperature, salinity, turbidity, pH and oxygen levels were measured during the study using a portable water profiler (Yeo-Kal Model 611, Yeo-Kal Electronics, Sydney). Three replicate measurements were taken monthly at each site for 8 months from June 2006 to February 2007 and the profiler was deployed at the same depth as the experimental frames (2 m). Data collected from the heavy metal analysis and physico-chemical variables were analysed with a three-factor nested ANOVA (see above) using a mean of all months taken for each site.

Principal components analysis was performed on the data collected for heavy metals and other physico-chemical variables in each of the estuaries (Fig. 2). Data were untransformed and subject to a varimax rotation to maximize the sum of the variances of the loading factors, resulting in a reduced number of factors contributing to the variances (Kaiser, 1958). Factors 1 and 2 explained 60% of the variance. Where several variables were highly correlated ( $r > 0.7$ ), the biologically redundant variable was omitted from regression analysis (Quinn & Keough, 2002). For example, where levels of a particular heavy metal were consistently below mean natural 'background' concentrations (values from Scanes & Roach, 1999) found in oysters, and therefore unlikely to have

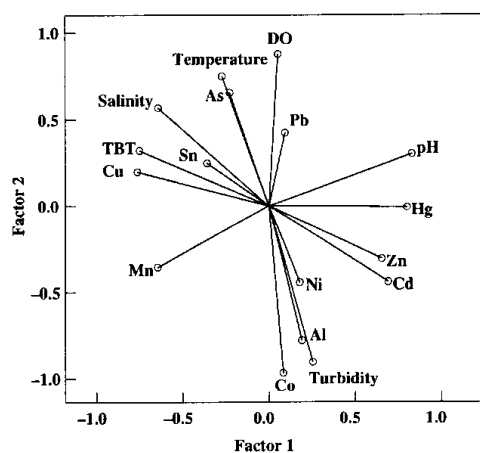


Figure 2 Principal Components Analysis using data collected for heavy metals and other physico-chemical variables in each of the estuaries.

a biological impact, they were omitted (As, Cd, Co, Mn; Table 1). To interpret the degree of heavy metal contamination at the different sites, levels of copper in our experimentally deployed oysters were compared with natural 'background' levels in uncontaminated oysters (Scanes & Roach, 1999), and levels of TBT with oyster shell deformity thresholds (Batley et al., 1992) and presented graphically. We then performed regression analyses on the remaining variables and percentage cover of the dominant fouling species.

SPSS was used to fit a line that best represented the data; in some cases, this was a curve. Regressions are presented graphically for  $r^2 \geq 0.3$ .

## RESULTS

Sixty taxa were identified during the study (most to genus or species) and of these, 17 were classified as native (eight of these have been recorded as invasive overseas and are therefore considered ES) and 25 as non-indigenous. The remaining 18 were classified as cryptogenic. The fauna included species of encrusting and arborescent bryozoans, barnacles, solitary and colonial ascidians, and serpulid polychaetes. The dominant taxa (those with an average percentage cover of > 5%) were used in univariate analyses and included the NIS, *Hydroides elegans* (Haswell, 1884), *Bugula neritina* (Linnaeus, 1758), *Watersipora subtorquata* (d'Orbigny, 1842), *Styela plicata* (Lesueur, 1823), *Botrylloides leachi* (Savigny, 1816) and *Diplosoma listerianum* (Milne-Edwards, 1841); the ES, *Amphibalanus variegatus* (Darwin, 1854), *Balanus trigonus* (Darwin, 1854), *Celleporaria nodulosa* (Busk, 1881) and *Pyura stolonifera* (Heller, 1878); and the native species, *Salmacina australis* (Haswell, 1884) (Table 2).

## Spatial variation in species distribution and diversity

Non-indigenous, native and cryptogenic species richness and percentage cover did not differ significantly between estuary types (Fig. 3). Instead, we found the strongest differences in species recruitment to be between individual estuaries and/or among sites rather than between commercial and recreational estuaries. Native species dominated space at nine of the 12

Table 1 Mean metal concentrations ( $\mu\text{g g}^{-1}$  dry weight) found in oysters deployed at three sites in four estuaries. Oyster replicates were lost from S2\* in Port Hacking and were replaced with the mean value of S1 and S3 from the same estuary.

Estuary	Site	As	Cd	Co	Cu	Mn	Zn	TBT ( $\text{ng g}^{-1}$ )
Observed concentrations								
Newcastle	S1	0.91	0.15	0.052	16.54	1.53	<b>470.05</b>	13
Newcastle	S2	1.25	0.11	0.048	11.12	2.26	<b>351.63</b>	12
Newcastle	S3	0.97	0.15	0.060	<b>25.40</b>	1.74	<b>528.74</b>	16
Port Kembla	S1	1.20	0.14	0.030	<b>40.54</b>	1.39	<b>494.96</b>	150
Port Kembla	S2	1.19	0.13	0.032	<b>40.71</b>	1.92	<b>419.23</b>	15
Port Kembla	S3	1.08	0.14	0.037	<b>101.20</b>	1.82	<b>531.06</b>	120
Port Hacking	S1	1.39	0.11	0.040	<b>54.79</b>	1.26	<b>474.44</b>	25
Port Hacking	S2*	1.41	0.11	0.038	<b>57.62</b>	1.36	<b>474.96</b>	45
Port Hacking	S3	1.42	0.11	0.036	<b>60.45</b>	1.45	<b>475.49</b>	64
Pittwater	S1	1.41	0.09	0.042	<b>88.48</b>	2.24	<b>437.93</b>	<b>86</b>
Pittwater	S2	1.11	0.10	0.045	<b>128.19</b>	2.40	<b>360.61</b>	<b>190</b>
Pittwater	S3	1.28	0.08	0.042	<b>126.76</b>	1.95	<b>421.34</b>	110
Background concentrations		(Scanes & Roach, 1999)						
Mean		1.88	0.54	0.064	21.6	2.53	277	(Batley et al., 1992) 84, 88, 107, 112 $\text{ng g}^{-1}$ – shell deformities present

Bold indicates values above natural 'background' concentrations or above levels known to cause shell deformities.

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Table 2 (a–c) Regression analyses ( $r^2$ -values) of species percentage cover and heavy metal loads. (d–e) Summary of binomial tests showing patterns among AF treatments, which occurred significantly more ( $P < 0.05$ ; pattern occurred on 13 frames of 18) than would be expected by chance in commercial and recreational estuaries. Species are classified as native (N) non-indigenous (NIS) and exported (ES; i.e. native to Australia, but listed as invasive in other parts of the world).

Classification	Species	(a) Cu			(b) TBT			(c) Zn			(d) Commercial estuaries				(e) Recreational estuaries			
		(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	CuDi	CuZnP	TBT	TBT	CuDi	CuZnP	TBT	TBT
ES (Qiani et al., 2007)	<i>Amphibalanus variegatus</i>	(–) 0.603*	(–) 0.531*	0.151	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ES (Qiani et al., 2007)	<i>Balanus trigonus</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NIS (Hutchings et al., 1989)	<i>Hydroides elegans</i>	0.494*	0.447*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
N (Haswell, 1884)	<i>Salmacina australis</i>	0.177	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NIS (Keough & Ross, 1999)	<i>Bugula neritina</i>	(–) 0.357*	(–) 0.713*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NIS (Keough & Ross, 1999)	<i>Watersipora subtorquata</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ES (Ingalls et al., 2006a,b)	<i>Celleporaria nodulosa</i>	0.275*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ES (Castilla et al., 2004)	<i>Pyura stolonifera</i>	(–) 0.258*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NIS (Hewitt et al., 2004)	<i>Borolyoides leachi</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NIS (Keough & Ross, 1999)	<i>Styela plicata</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NIS (Hewitt et al., 2004)	<i>Diplosoma listerianum</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

(a–c) Value indicates where these regressions represent a significant relationship between the variables ( $P < 0.05$ ; \* $P < 0.01$ ). (–) No significant patterns. (–) Relationship between species percentage cover and heavy metal loads was negative.

†Response to paint treatment opposite to relationship with background metal levels.

‡Response to paint treatment similar to relationship with background metal levels.

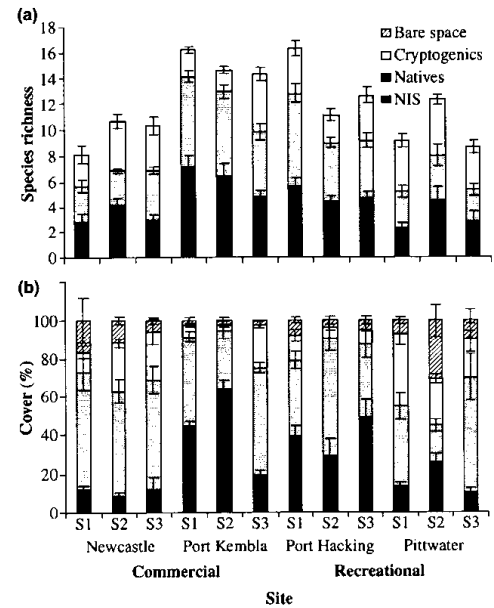


Figure 3 Species richness and percentage covers ( $\pm$ SE) of non-indigenous, native and cryptogenic species (and bare space) collected on settlement plates deployed at three sites within estuaries of commercial or recreational vessel activity.

sites (Fig. 3), and this native dominance of space can be attributed primarily to *A. variegatus* in Newcastle (40–55%), *Salmacina australis* in Port Kembla and Port Hacking (10–35%) and *C. nodulosa* in Pittwater (10–50%). Mean numbers of native and non-indigenous species were strongly positively correlated ( $r = 0.693$ ; Fig. 4), ranging from  $2.5 (\pm 0.4)$  to  $7.1 (\pm 0.7)$  for natives and  $2.3 (\pm 0.3)$  to  $7.2 (\pm 0.9)$  for NIS (Fig. 3).

The exported barnacles, *A. variegatus* and *B. trigonus*, were the major space occupiers in the study and their percentage covers varied between individual estuaries ( $F_{2,8} = 69.31$ ,  $P = 0.000$  and  $F_{2,8} = 10.38$ ,  $P = 0.006$  respectively), but not between estuary types (i.e. commercial versus recreational). The non-indigenous serpulid polychaete, *H. elegans*, occupied more space on plates deployed in one commercial port (Port Kembla) than another (Newcastle) ( $F_{2,8} = 28.55$ ,  $P = 0.000$ ). Percentage cover of *H. elegans* also differed significantly between the two recreational estuaries, while the native serpulid, *S. australis* varied in its abundance among sites within one of the recreational estuaries (Port Hacking; SNK,  $P < 0.05$ ). Percentage covers of the non-indigenous bryozoans, *B. neritina* and *W. subtorquata*, and the exported bryozoan, *C. nodulosa*, varied among sites within estuaries ( $F_{8,60} = 3.42$ ,  $P = 0.003$ ;  $F_{8,60} = 3.62$ ,  $P = 0.002$  and  $F_{8,60} = 3.96$ ,  $P = 0.000$  respectively). *W. subtorquata* was generally absent from all sites

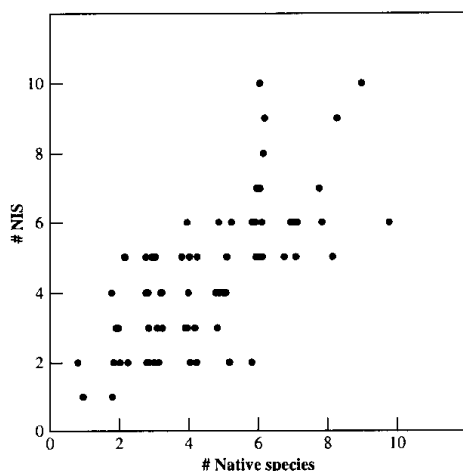


Figure 4 Correlation plot between non-indigenous and native species richness.

apart from in Port Hacking where percentage cover was between 10 and 20%. Percentage cover of the exported ascidian, *P. stolonifera*, and the non-indigenous ascidian, *B. leachi*, varied significantly between the two recreational estuaries ( $F_{2,8} = 5.68$ ,  $P = 0.005$  and  $F_{2,8} = 7.05$ ,  $P = 0.017$  respectively), but not between commercial estuaries. In contrast, the non-indigenous solitary ascidian, *S. plicata* showed variation in its recruitment among sites in Port Kembla ( $F_{8,60} = 8.68$ ,  $P = 0.000$ ). The colonial ascidian, *D. listerianum*, was the only NIS to differ significantly between estuary type and occupied more space on plates in commercial than in recreational estuaries ( $F_{1,2} = 33.75$ ,  $P = 0.028$ ).

#### Heavy metals in oyster tissue

Heavy metal levels were also highly variable among sites but did not differ significantly between commercial and recreational estuaries. Levels of Cu (in all but two commercial sites;  $F_{8,24} = 3.28$ ,  $P = 0.011$ ; Fig. 5) and Zn (at all sites;  $F_{8,24} = 0.471$ ,  $P = 0.864$ ) were found to be well above natural 'background' concentrations (Table 1). At site 3 in Port

Kembla (commercial) and all sites in Pittwater (recreational), Cu levels were three times greater ( $88\text{--}128\ \mu\text{g g}^{-1}$ ) than 'natural' background concentrations ( $21.6\ \mu\text{g g}^{-1}$ ) (Fig. 5). Zn levels were almost double ( $350\text{--}530\ \mu\text{g g}^{-1}$ ) the levels that would be expected in uncontaminated oyster tissue ( $277\ \mu\text{g g}^{-1}$ ) at all sites in the study (Table 1). TBT levels were the highest in Pittwater and Port Kembla ( $F_{2,8} = 4.85$ ,  $P = 0.042$ ) and were at levels that could potentially cause shell deformities (Table 1; Fig. 5), although no deformities were observed in the experimental oysters.

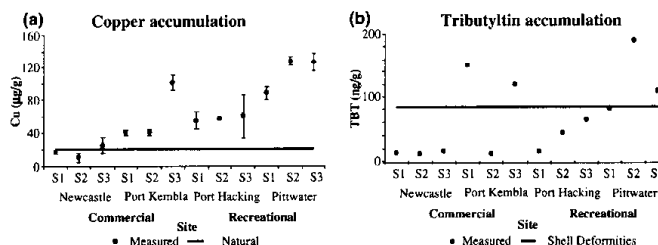
#### Relationships between species percentage cover and heavy metal loads

Six species were strongly related to heavy metal loads, including five invaders and one native species. Percentage cover of the exported barnacle, *A. variegatus*, was negatively related to levels of Cu and TBT and showed a weak positive relationship with Zn (Table 2a–c; Fig. 6a,b). *A. variegatus* also responded positively to the TBT paint treatment, but only at commercial sites (Table 2d). Percentage cover of *B. trigonus* (exported) was not related to background levels of Cu, TBT or Zn (Table 2a–c), and was reduced on all the AF treatments in commercial estuaries (Table 2d).

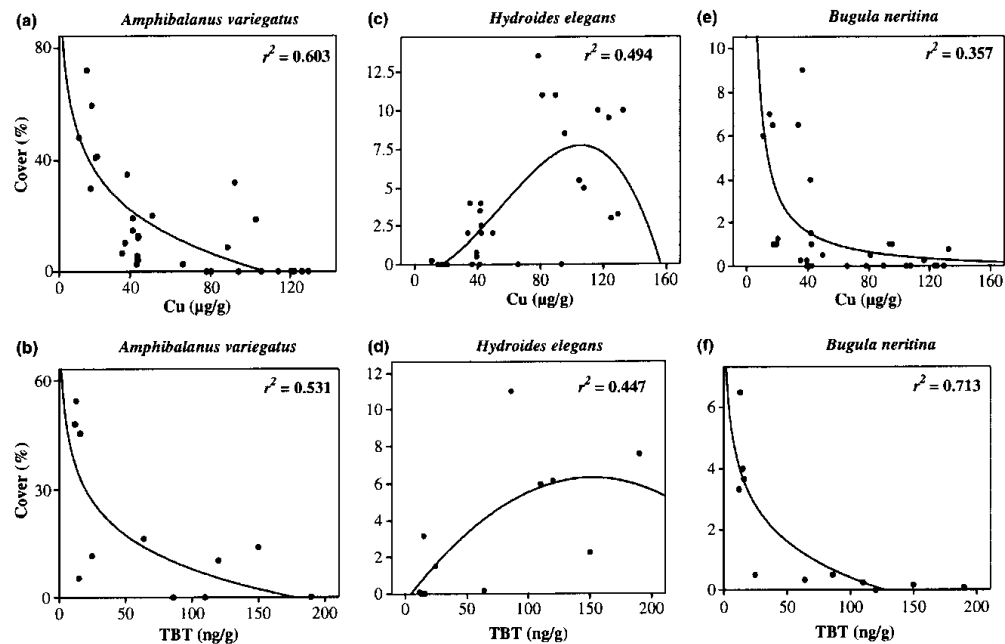
The serpulids *H. elegans* (non-indigenous) and *S. australis* (native) showed similar patterns in their percentage covers between estuaries and sites, and these were positively related to background levels of Cu (both species) and TBT (*H. elegans* only) (Table 2a,b; Fig. 6c,d). *S. australis* and *H. elegans* also showed a positive response to experimentally applied CuZnP, CuDi (*S. australis*) or TBT paints (*H. elegans*) (Table 2e), but only at the sites where background Cu/TBT levels were already elevated (generally recreational sites).

The non-indigenous bryozoan, *B. neritina*, was more patchily distributed and its percentage cover was negatively related to levels of Cu and TBT (Table 2a,b; Fig. 6e,f), but showed no response to the AF treatments (Table 2d,e). In contrast, percentage cover of the non-indigenous bryozoan, *W. subtorquata*, was not related to background Cu or TBT levels (Table 2a,b), but did show an effect of the AF treatments. Specifically, the percentage cover of *W. subtorquata* increased on CuDi plates compared with control plates (significant at commercial sites; Table 2d). Percentage cover of the exported *C. nodulosa* was positively related to increasing

Figure 5 Comparison of copper ( $\mu\text{g g}^{-1}$  dry weight) and tributyltin ( $\text{ng g}^{-1}$  dry weight) concentration in oysters experimentally deployed at three sites within estuaries of commercial or recreational activity. Three replicates per site are reported for (a) copper accumulation and one replicate per site for (b) tributyltin accumulation.



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Figure 6 Regression analyses of species percentage cover and heavy metal loads. Results are presented for  $r^2 > 0.3$ .

background Cu levels (Table 2a) and the bryozoan responded negatively to the CuZnP and TBT paint treatments (Table 2e).

The exported ascidian, *P. stolonifera*, was less abundant at sites with high background Cu levels (Table 2a) and its percentage cover was negatively affected by TBT on plates at most commercial sites, but not at recreational sites (Table 2d,e). The non-indigenous ascidians, *B. leachi*, *S. plicata* and *D. listerianum*, showed no relationship with background heavy metal loads and no effect of the AF treatments.

#### Relationships between species percentage cover and physico-chemical variables

Nine species were strongly related to changes in physico-chemical variables, including eight invaders and one native species. Of these variables, DO and salinity varied between estuaries ( $F_{2,8} = 46.55$ ,  $P = 0.000$  and  $F_{2,8} = 16.03$ ,  $P = 0.002$  respectively). Temperature, turbidity and pH varied between sites within estuaries ( $F_{8,24} = 31.54$ ,  $P = 0.000$ ;  $F_{8,24} = 5.38$ ,  $P = 0.001$  and  $F_{8,24} = 13.50$ ,  $P = 0.000$  respectively).

The barnacle, *A. variegatus*, was negatively related to DO, salinity and temperature, and positively related to turbidity (Table 3; Fig. 7a–c) while percentage cover of *B. trigonus* increased with increasing DO, temperature and pH (Table 3; Fig. 7d,e). Percentage cover of *H. elegans* was positively related to increasing salinity (Table 3; Fig. 7f), and increased percent-

age cover of *H. elegans* and *S. australis* was also related to increased temperatures (Table 3; Fig. 7g). *H. elegans* and *S. australis* were negatively related to pH and turbidity respectively (Table 3). Percentage covers of the bryozoans, *B. neritina* and *W. subtorquata*, were also related to turbidity, positively and negatively respectively (Table 3; Fig. 7h), and *C. nodulosa* was negatively related to pH (Table 3; Fig. 7i). The solitary ascidian, *P. stolonifera*, was not related to the measured physico-chemical variables while the colonial ascidian, *B. leachi*, was positively correlated with pH (Table 3; Fig. 7j). Percentage cover of *S. plicata* also increased with increasing DO and pH and decreasing turbidity (Table 3; Fig. 7k,l).

#### DISCUSSION

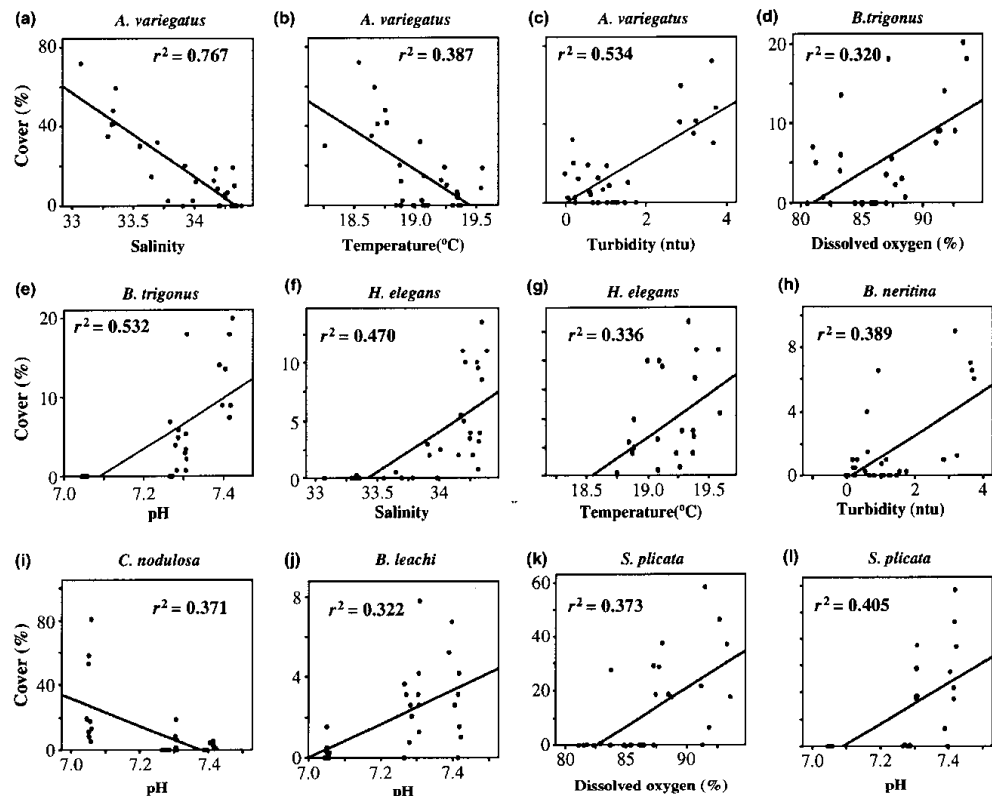
Estuaries are among the most highly disturbed marine environments receiving large inputs of contaminants and high volumes of shipping traffic, and as such provide an ideal environment for invasive species to establish and persist (Carlton, 1996b; Piola & Johnston, 2007). We compared recruitment of sessile invertebrates in four impacted estuaries (recreational or commercial vessel activity) with differing contaminant loads and physico-chemical conditions, as well as the effect of different AF paint treatments on recruitment. This study has highlighted the potential role of metal pollution and physico-chemical variables in the establishment of invasive fouling species in new regions. We found that levels of



**Table 3** Regression analyses ( $r^2$ -values) of species percentage cover and physico-chemical variables.

Species	(a) Dissolved Oxygen (%)	(b) Salinity	(c) Temperature (°C)	(d) Turbidity (ntu)	(e) pH
<i>Amphibalanus variegatus</i>	(-) 0.285*	(-) 0.767*	(-) 0.387*	0.534*	—
<i>Balanus trigonus</i>	0.320*	—	0.162	—	0.532*
<i>Hydroides elegans</i>	—	0.470*	0.336*	—	(-) 0.297*
<i>Salmacina australis</i>	—	—	0.204	(-) 0.141	—
<i>Bugula neritina</i>	—	(-) 0.268*	(-) 0.207	0.389*	—
<i>Watersipora subtorquata</i>	—	—	—	(-) 0.218	—
<i>Celleporaria nodulosa</i>	—	—	—	—	(-) 0.370*
<i>Pyura stolonifera</i>	—	—	—	—	—
<i>Botrylloides leachi</i>	—	—	—	—	0.322*
<i>Styela plicata</i>	0.373*	—	—	(-) 0.275*	0.405*
<i>Diplosoma listerianum</i>	—	—	—	—	—

Value indicates where these regressions represent a significant relationship between the variables ( $P < 0.05$ , \* $P < 0.01$ ). —No significant patterns.

**Figure 7** Regression analyses of species percentage cover and physico-chemical variables. Results are presented for  $r^2 > 0.3$ .

anthropogenic impacts (in the form of heavy metal contamination) and natural physico-chemical variables were more important correlates of species distributions (including 10 invaders and one native species) than the dominant estuary

type (recreational versus commercial). This has implications for the management of vectors as well as ports and marinas, which may act as sources of propagules for invasive species.

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Non-indigenous species can be more abundant in estuaries than on open coast (Wasson *et al.*, 2005) and have also been found in estuaries that lack commercial shipping (Wasson *et al.*, 2001; Wyatt *et al.*, 2005). Cohen *et al.* (2005) sampled several different habitats within port and non-port areas and found no difference in NIS richness between them; however, their results could have been influenced by the rapid assessment methodology and the likelihood of different substrate types (Glasby, 2000), ages of assemblages (Glasby, 1999) and sample sizes at each location. Our study controlled the age of assemblage and substrate composition, but substrata in commercial estuaries were stationary, while those in recreational estuaries were moving. We found that the dominant taxa differed in their percentage covers between estuaries and sites, but this was not related to estuary type (i.e. recreational versus commercial), except for the non-indigenous ascidian *D. listerianum*, which was more prevalent in commercial estuaries. Recreational estuaries had just as many NIS as commercial estuaries in spite of the differences in international vessel traffic, a result similar to that in Wyatt *et al.* (2005). Native species were found to dominate space in nine of the 12 sites. Numbers of non-indigenous and native species were strongly positively correlated with each other, which agree with findings from other large-scale experimental studies of invasion (Lonsdale, 1999; Stohlgren *et al.*, 1999; Levine, 2000; Stohlgren *et al.*, 2003; Huston, 2004; Davis *et al.*, 2000). Our comparison between commercial and recreational estuaries may not have detected differences for some species because of the structures available for our experimental plates. The invertebrate larvae of many fouling species are known to disperse short distances from the adult populations (Keough, 1983) and therefore the relationships between species abundances and metals or physico-chemical variables, which occurred at smaller spatial scales (between sites, 1–3 km apart), may be more important considerations in the development of fouling assemblages.

We found levels of copper and zinc to be well above 'natural' background levels in 10 of 12 sites (Scanes & Roach, 1999). Tributyltin contamination was also found to be a substantial problem in large recreational marinas (with levels that could potentially result in shell deformities in oysters) despite a ban on its application on boats < 25 m since 1989 and recent studies suggesting that TBT contamination is no longer a problem in port areas (Dowson *et al.*, 1993; Evans *et al.*, 1995, but see; Gibson & Wilson, 2003). TBT chemistry is largely dependent on local environmental conditions and its partitioning between dissolved and particle-adsorbed states depends on factors such as particle concentration and organic carbon content, salinity and pH (Harris *et al.*, 1996). While TBT has a half-life of a few days in the water column (Seligman *et al.*, 1996), in sediments it is thought to vary considerably both spatially and temporally, potentially in the order of months to years (Harris *et al.*, 1996). In highly turbid areas, the TBT uptake potential of sediments is increased (Harris *et al.*, 1996), which may partially explain why TBT levels were lower in the water column in the well-flushed and highly turbid waters of the commercial Newcastle Harbour

than in the more sheltered recreational marinas (Floerl & Inglis, 2003).

Heavy metals have long been recognized as important selection agents acting on aquatic organisms (reviewed by Klerks & Weis, 1987). In polluted areas, organisms are under selective pressure for increased resistance to toxicants. This can result in physiological acclimation as tolerance is gained through exposure to sublethal concentrations, or the evolution of genetically based resistance through natural selection (Klerks & Weis, 1987; Levinton *et al.*, 2003). Several physiological mechanisms are recognized for coping with metal stress including the release of extracellular metabolites, which bind to metals and reduce the metal concentration surrounding the organism (algae: Fogg & Westlake, 1955; McKnight & Morel, 1979; Fisher & Fabris, 1982). Some invertebrates also exhibit sequestering mechanisms such as the binding of metals to inducible metallothioneins (Olafson *et al.*, 1979; Suzuki *et al.*, 1980; Thompson *et al.*, 1982; Engel & Brouwer, 1986; Jenkins & Sanders, 1986; Roesijadi, 1986) or in granules and vesicles (Brown, 1977; George & Pirie, 1979; Lowe & Moore, 1979; Mason *et al.*, 1984).

The NIS *H. elegans* responded positively to the copper AF treatments and dominated in areas where background copper levels were high. *H. elegans* is generally thought of as a copper-tolerant species (Allen, 1953; Johnston & Keough, 2003; Piola & Johnston, 2007; Dafforn *et al.*, 2008), and exhibits tolerance of TBT (this study), which together may have aided its cosmopolitan dispersal on vessel hulls (Pettengill *et al.*, 2007) and establishment in polluted harbours around the world since the 1800s (Ruiz *et al.*, 2000). We have also demonstrated the potential for several native species to thrive in conditions of elevated copper (*S. australis* and *C. nodulosa*) (but see also Piola & Johnston, 2007; Dafforn *et al.*, 2008) or TBT (*S. australis*). Five native species also responded positively to the anti-fouling paints (*A. variegatus* and *S. australis*) or exhibited a tolerance (*B. trigonus* and *Pyura stolonifera* – recreational estuaries and *C. nodulosa* – commercial estuaries and CuDi in recreational estuaries). Metal tolerance therefore has the potential to advantage some native species within both donor and recipient ports and harbours that have elevated metal levels and a less tolerant resident biota, thereby increasing their chances of being exported overseas on hulls painted with copper- or TBT-based AF paint. With the exception of *S. australis*, these are all ES with introduced ranges of Japan [*A. variegatus*, *B. trigonus*; (Otani *et al.*, 2007)], New Zealand [*C. nodulosa*; (Inglis *et al.*, 2006a,b)] and Chile [*P. stolonifera*; (Castilla *et al.*, 2004)]. Japan, along with China, USA, South Korea and New Zealand are the major destinations for Australian maritime trade (BTRE 2007). The high (and increasing) volume of trade between these areas represents a significant risk of invasion from Australian species that have been exported. Interestingly, *S. australis* is recognized as endemic to Australia, but its high tolerance to copper, particularly in recreational estuaries, suggests its potential for export around the globe. Combining information about shipping routes with information about species metal

tolerances could enhance predictions about potential exports to other regions.

Physico-chemical variables including pH, temperature and turbidity were also important predictors of the distribution of fouling species and in three instances, explained > 50% of the variation in species percentage cover. Percentage cover of *A. variegatus* increased with increasing turbidity, while percentage cover of the solitary ascidian, *Styela plicata*, decreased. Many ascidians are negatively affected by suspended sediment. For example, the solitary ascidians, *Ciona intestinalis* and *Ascidella aspersa*, are sensitive to inorganic material in the water column, which reduces their filter feeding efficiency and can lead to reduced growth rates and mortality (Robbins, 1985). Excessive sediment can also result in burial and clogging of ascidian siphons and branchial structures (Bakus, 1968). Potentially the increased sediment load in the water column inhibits growth of ascidian recruits, causing mortality and reducing competition for space with other taxa such as *A. variegatus*. Indirect benefits of reduced ascidian densities have been recorded previously for barnacles, bryozoans and serpulids (Johnston & Keough, 2003).

pH was also strongly correlated with percentage cover of five invaders. Mean decreases in pH of 0.4 units were related to ~20% decreases in percentage cover of *B. trigonus* and *S. plicata*. In the future, the increased sequestration of carbon dioxide by the ocean as a consequence of global warming is expected to lower pH levels with realistic changes in the order of 0.5 pH (The Royal Society 2005). This could have a direct effect on the physiology of marine organisms (particularly calcifying species, e.g. barnacles). Increased ocean acidification may also have indirect effects on marine organisms by modifying the chemistry of toxins such as trace metals and increasing bioavailability (The Royal Society 2005). Similarly, predicted temperature increases of 1.8–4 °C by the year 2100 (IPCC 2007) could enhance toxic effects of metals (reviewed by Sokolova & Lannig, 2008). This can partly be explained by increased metabolic activity that results in higher uptake, but also the bioavailability of metals increases at higher temperatures as a result of the increased solubility of metal compounds. Interestingly, several species that were correlated with copper and TBT, including *H. elegans*, *S. australis*, *A. variegatus* and *B. neritina*, showed a similar relationship with temperature, suggesting potential interactive effects of these physico-chemical variables. Future increases in metal bioavailability through ocean acidification and global warming may further advantage metal-tolerant species and result in their increased dominance of polluted areas where they can act as a propagule source for further export.

There is a widespread trend to reduce heavy metal contamination of estuaries and ports (Minchin & Gollasch, 2003). TBT contamination has diminished (Evans et al., 1995), but copper, which is still commonly used in AF paints, is accumulating in estuaries and having toxic and sublethal effects on marine species (Claisse & Alzieu, 1993; Hall et al., 1998). Minchin & Gollasch (2003) suggested that remediation of harbours might lead to increased potential for NIS to

invade and establish. We would predict the opposite based on this study and the work by Piola & Johnston (2007), which suggest that reducing pollution loads in harbours could increase the resilience of native communities and reduce the dominance of invaders thereby reducing the number of invasive propagules available for export. One method for reducing metal loads in ports and harbours would involve legislation to enforce the use of non-toxic AF strategies. There currently exist several non-toxic alternatives to copper on the market and new technologies are in the process of being developed to replace harmful biocides in AF paints (Srinivasan & Swain, 2007). However, if these alternatives prove less effective at preventing biofouling, then we risk increasing the transfer rate of NIS.

Research points to the importance of the precautionary principle when dealing with NIS (Floerl et al., 2005), that is, every introduction should be considered potentially harmful. Past eradication attempts have proven costly and difficult (Willan, 2000; Anderson, 2005; Coutts & Forrest, 2007) and many current management programmes aim to prevent the arrival of new pests. To this end, donor region and vector management are essential. Our results suggest that recreational estuaries are just as likely to harbour invaders as commercial estuaries subject to extensive international vessel operations (see also Wasson et al., 2001) and their dominance is often related to metal loads and the prevailing physico-chemical conditions. Of particular concern is how the effect of metal contamination might be magnified by a changing climate. To this end, it is important for us to consider the potential for increased temperature and ocean acidification to increase metal bioavailability and thereby increase the vulnerability of ports and estuaries to invasion.

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**Appendix II:** List of species found during thesis and their classification status as native (N), non-indigenous (NIS), exported (ES) and cryptogenic (C). *D. listerianum* has been classified as NIS or C by different authors and in this study was treated as NIS following the reasoning of Ruiz et al. (2000).

Phylum	Status (N/NIS/ES/C)	Native Distribution	Invasive Distribution	Source
<b>ANNIMALIA</b>				
<b>Arthropoda</b>				
<i>Amphibalanus variegatus</i>	ES	Indo-West Pacific (Including Australia and New Zealand)	Japan	Darwin (1854); Glasby et al. (2007); Otani (2007)
<i>Amphibalanus amphitrite</i>	NIS	Indo-West Pacific	Cosmopolitan	Darwin (1854); Ruiz et al. (2000), Hewitt et al. (2004)
<i>Balanus trigonus</i>	ES	Indo-West Pacific (Including Australia)	Hawaii	Darwin (1854); Glasby et al. (2007); Ruiz et al. (2000)
<i>Megabalanus coccopoma</i>	NIS	SE Pacific	NE Atlantic, S Atlantic, Australia	Darwin (1854); (USGS 2008d)
<i>Austrobalanus imperator</i>	N	Australia		Darwin (1854); Glasby et al. (2007)



Phylum	Status (N/NIS/ES/C)	Native Distribution	Invasive Distribution	Source
<b>Cnidaria</b>				
Hydroid 1	C			
Hydroid 2	C			
Hydroid 3	C			
<b>Echinodermata</b>				
<i>Anthenea sidneyensis</i>	N	Australia		Doderlein (1915)
<i>Centrostephanus rogersii</i>	ES	NSW, Australia	Tasmania, Australia	Ling (2009)
<b>Ectoprocta</b>				
<i>Arachnopusia unicomis</i>	N	Australia and New Zealand		Hutton (1873); Glasby et al. (2007)
<i>Beania magellanica</i>	N	Southern Ocean including Australia, New Zealand, South America, South Africa. (Cryptogenic in NW Pacific and NE Atlantic)		Busk (1852); Glasby et al. (2007)
<i>Bowerbankia gracilis</i>	NIS	W Atlantic	Cosmopolitan	Ruiz et al. (2000); Keough & Ross (1999)
<i>Bugula dentata</i>	ES	Indo- West Pacific including Australia (cryptogenic in Hawaii, S Pacific Islands South Africa)		Lamouroux (1816); (USGS 2008a)

Phylum	Status (N/NIS/ES/C)	Native Distribution	Invasive Distribution	Source
<i>Bugula flabellata</i>	NIS	NE Atlantic	Cosmopolitan	Keough & Ross (1999); Hewitt et al. (2004)
<i>Bugula neritina</i>	NIS	NE Atlantic	Cosmopolitan	Keough & Ross (1999); Ruiz et al.(2000); Hewitt et al. (2004)
<i>Bugula stolonifera</i>	NIS	Southern Britain	Cosmopolitan	Keough & Ross (1999) (USGS 2008b)
<i>Celleporaria nodulosa</i>	ES	Australia	New Zealand	Busk (1881); Aqueal Pty Ltd (2002); Inglis et al.(2006a, b)
<i>Conopeum seurati</i>	NIS	Mediterranean and NE Atlantic	Cosmopolitan	Keough & Ross (1999); Hewitt et al. (2004)
<i>Crisia acropora</i>	N	Australia		Busk (1852)
<i>Cryptostula pallasiana</i>	NIS	N Atlantic	Cosmopolitan	Keough & Ross (1999) (NIMPIS 2002)
<i>Fenestulina mutabilis</i>	N	Australia		Hastings (1932); Glasby et al. (2007)
<i>Microporella umbracula</i>	NIS	Mediterranean, W Atlantic and Indo-Pacific?	Australia?	Audouin (1826)

Phylum	Status (N/NIS/ES/C)	Native Distribution	Invasive Distribution	Source
<i>Reteporella</i> sp.	C			
<i>Savignyella lafonti</i>	C			
<i>Schizomavella</i> sp.	C			
<i>Schizoporella errata</i>	NIS	Mediterranean	Cosmopolitan	Keough & Ross (1999); Hewitt et al. (2004)
<i>Tricellaria inopinata</i>	NIS	NE and NW Pacific	Cosmopolitan	Keough & Ross (1999); Hewitt et al. (2004)
<i>Watersipora arcuata</i>	NIS			
<i>Watersipora subtorquata</i>	NIS	E Pacific	Cosmopolitan	Ruiz et al. (2000)
<b>Mollusca</b>				
<i>Mytilus galloprovincialis</i> <i>planulatus</i>	N	Australia		Beu (2004); Daguin (2000); Hilbish (2000)
<i>Saccostrea commercialis</i>	N	Australia		Glasby et al. (2007)
<b>Polychaeta</b>				
<i>Ficopomatus enigmatus</i>	NIS	Unkown	Cosmopolitan	Keough & Ross (1999)
<i>Galeolaria caespitosa</i>	N	Australia		Mörch (1863); Glasby et al. (2007)
<i>Hydroides deleoni</i>		Unkown	Cosmopolitan	

Phylum	Status (N/NIS/ES/C)	Native Distribution	Invasive Distribution	Source
<i>Hydroides elegans</i>	NIS	Unknown	Cosmopolitan	Haswell (1884); Hewitt et al. (2004)
<i>Hydroides ezoensis</i>	NIS	Unknown	Cosmopolitan	(Hewitt 2002)
<i>Hydroides diramphus</i>	NIS		Australia	Mörch (1863); (AMBS 2002)
<i>Pomatocerus taeniata</i>	N	Australia		Lamarek (1818); Aqueenal Pty Ltd (2002, 2004)
<i>Salmacina australis</i>	N	Australia		Haswell (1884); Aqueenal Pty Ltd (2002, 2004)
Spirorbidae	C			
Sabellidae	C			
<b>Porifera</b>				
<i>Sycon</i> sp.	C			
Sponge 1	C			
Sponge 2	C			
<b>Tunicata</b>				
Ascidian 1	C			

Phylum	Status (N/NIS/ES/C)	Native Distribution	Invasive Distribution	Source
Ascidian 2	C			
<i>Botrylloides magnicoecum</i>	N	Australia		Hartmeyer (1912); Pollard and Pethebridge (2002a, b)
<i>Botrylloides leachi</i>	NIS	NW Pacific	Cosmopolitan	Hewitt et al (2004)
<i>Botryllus schlosseri</i>	NIS	NW Atlantic, NE Pacific, Australia, New Zealand	Cosmopolitan	Hewitt et al. (2004)
<i>Didemnum</i> spp.	C			
<i>Diplosoma listerianum</i>	NIS	NW Pacific	NW Atlantic, NE Atlantic, Lambert & Lambert (1998); Hawaii, NE Pacific, Australia, New Zealand	Ruiz et al. (2000)
<i>Herdmania momus</i>	ES	Australia	Hawaii	Savigny (1816); Pollard and Pethebridge (2002b, a); (USGS 2008c)
<i>Perophora japonica</i>	NIS	Japan, Korea	Cosmopolitan	(Lambert 2005)
<i>Pyura ganglia</i>	N	Australia		(Nishikawa et al. 2000)
				Glasby et al. (2007)

Phylum	Status (N/NIS/ES/C)	Native Distribution	Invasive Distribution	Source
<i>Pyura stolonifera</i>	ES	Australia	Chile	(Castilla et al. 2004)
<i>Styela plicata</i>	NIS	East Asian Seas	Caribbean, S Atlantic, NE Kott (1985); Keough & Ross Pacific, NW Pacific, (1999) Australia	
<b>PLANTAE</b>				
<b>Phaeophyta</b>				
<i>Colpomenia sinuosa</i>	NIS	Unknown	Cosmopolitan	(Lewis 1999)
<i>Dictyota dichotoma</i>	NIS	Unknown	Cosmopolitan	Lewis (1999)
<i>Ecklonia radiata</i>	N	Australia		Glasby et al. (2007)
<i>Padina fraseri</i>	N	Australia		Glasby et al. (2007)
<i>Sargassum</i> sp.	N	Australia		Glasby et al. (2007)
<b>Rhodophyta</b>				
<i>Corallina</i> sp.	N	Australia		Glasby et al. (2007)

**Appendix III:** Full ANOVA tables for Chapter 2

Analyses of variance comparing percent covers of non-indigenous and native species on settlement plates that were deployed at 3 sites within estuaries of commercial or recreational vessel activity.

% cover		Non-indigenous species				Native species		
Source	DF	MS	F	P	MS	F	P	
Estuary Type	1	1.18	0.02	0.905	1058	1.10	0.404	
Es (EsTy)	2	63.86	8.31	<b>0.011</b>	961.26	0.83	0.471	
Si (Es(EsTy))	8	7.68	5.78	<b>0.000</b>	1159.46	4.09	<b>0.001</b>	
Error	60				283.32			

**Bold** indicates significantly different at  $P < 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .

Analyses of variance comparing percent covers of individual species on settlement plates that were deployed at 3 sites within estuaries of commercial or recreational vessel activity.

% cover		<i>Amphibalanus variegatus</i>			<i>Balanus trigonus</i>			<i>Hydroides elegans</i>			<i>Salmacina australis</i>			<i>Bugula neritina</i>		
Source	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Estuary Type	1	9248.00	1.20	0.388	171.13	0.25	0.666	5.12	0.29	0.646	425.35	0.67	0.431	ln(x+1)		
Es (EsTy)	2	7736.11	69.31	<b>0.000</b>	680.68	10.38	<b>0.006</b>	17.85	28.55	<b>0.000</b>	706.57	1.12	0.364	10.30	2.39	0.262
Si (Es(EsTy))	8	69.40	0.62	0.757	65.56	1.60	0.145	0.63	2.10	0.049	612.67	7.67	<b>0.000</b>	4.30	4.28	0.055
Error	60	117.24			41.03			0.30			79.90			1.01	3.42	<b>0.003</b>
														0.29		

% cover		<i>Pyura stolonifera</i>			<i>Botrylloides leachi</i>			<i>Styela plicata</i>			<i>Diplosoma listerianum</i>			<i>Celleporaria nodulosa</i>		
Source	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Estuary Type	1	11.68	0.65	0.506	0.20	0.03	0.872	138.89	0.03	0.871	3.80	10.56	<b>0.002</b>	ln(x+1)		
Es (EsTy)	2	18.06	5.68	<b>0.005</b>	6.00	7.05	<b>0.017</b>	4090.45	4.51	<b>0.049</b>	0.11	0.31	0.732	5287.35	2.10	0.285
Si (Es(EsTy))	8	3.29	1.04	0.418	0.85	2.03	0.058	906.91	8.68	<b>0.000</b>	0.40	1.11	0.369	2522.90	3.98	0.063
Error	60	3.16			0.42			104.42			0.36			634.11	3.96	<b>0.001</b>
														160.32		

**Bold** indicates significantly different at P < 0.05. *Post-hoc* pooling done at P > 0.25.



**Appendix IV:** Dafforn, K. A., E. L. Johnston, and T. M. Glasby. 2009. Shallow moving structures promote marine invader dominance. *Biofouling* **25**:277-287.

### Shallow moving structures promote marine invader dominance

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Global increases in urban development have resulted in severe habitat modification in many estuaries. Most are now dominated by artificial structures, which might have a myriad of effects on native species. The provision of extra hard substrata presents additional free space, and recent research suggests non-indigenous epifauna may be able to exploit these artificial structures (particularly pontoons) more effectively than native species. The early development of fouling assemblages was compared on settlement plates attached to fixed or moving experimental structures at depths of 0.5 m and 2 m. Invertebrate invaders as a group were disproportionately more numerous on shallow, moving plates (essentially floating surfaces) than on deeper plates, whereas native epifauna were less numerous than invaders in all treatments. Importantly, however, individual invasive species showed differing effects of movement and depth. Future management strategies should take into account the potential for shallow, moving structures to enhance invader dominance and strongly consider using fixed structures to reduce opportunities for invaders.

**Keywords:** non-indigenous species; floating; pontoons; artificial structures

#### Introduction

The estuarine environment faces increasing pressure from encroaching urban developments. The addition of artificial structures such as seawalls, pilings and pontoons to urban waterways is of particular concern because of the potential effect of these structures on sessile epifauna. Comparisons of artificial structures and natural reefs in sheltered parts of Sydney Harbour have revealed distinct differences in the assemblages able to colonise and persist on these structures vs. natural reefs. Intertidal assemblages on seawalls tend to be distinct from those on rocky reefs, supporting fewer species and being dominated by algae (Bulleri and Chapman 2004). Subtidally, assemblages on sandstone seawalls and reefs are also distinguished from pilings and pontoons by fewer taxa and a greater cover of coralline algae (Connell and Glasby 1999). Moreover, pilings and pontoons tend to support more bryozoans, ascidians and mussels than rocky reefs (Glasby 1999a; Connell 2001). Differences between artificial structures and natural rocky reefs arise due to their physical characteristics, including substratum composition (Anderson and Underwood 1994, Glasby 2000; Chapman and Bulleri 2003), age (Perkol-Finkel et al. 2005), orientation (Connell 1999; Glasby and Connell 2001; Saunders and Connell 2001; Knott et al.

2004), predation levels (Nydam and Stachowicz 2007), illumination levels (Glasby 1999b) and due to the fixed nature of pilings, seawalls and reefs vs. the movement of pontoons (Connell 2000; Holloway and Connell 2002; Neves et al. 2007; Perkol-Finkel et al. 2008; Shenkar et al. 2008).

Association with artificial structures is used as a criterion for identifying non-indigenous species (NIS) (Chapman and Carlton 1991). However, the influence of these structures on invasion is still not fully understood. NIS can be a significant threat to ecosystems, as they have the potential to reduce native biodiversity through competition for resources, habitat alteration or predation (Mack et al. 2000; Holloway and Keough 2002). Ports and estuaries are major foci for arriving invaders and contain a large source population for further transport and dispersal (Carlton 1987; Floerl and Inglis 2005).

The addition of artificial boating structures to estuaries may assist the establishment of invaders by creating space, a major limiting resource for hard-substratum assemblages (Sutherland 1974; Buss 1979; Russ 1982). Such structures also provide a greater diversity of habitat, both moving and fixed, which invading species may be able to exploit more effectively than native species (Glasby et al. 2007). Bulleri and Airoidi (2005) found that invasion of seawalls by the

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alga *Codium fragile* ssp. *tomentosoides* was controlled by both disturbance (in the form of wave exposure) and biotic resistance from the superior native space occupier *Mytilus galloprovincialis*. Vaselli et al. (2008) highlight the potential for seawalls to alter water circulation patterns and aid the establishment of invasive species. Similarly, Glasby et al. (2007) found the greatest number of NIS on pontoons and the least on seawalls and rocky reefs where native species dominated space. Pilings and pontoons are of particular concern due to their increasing presence in boating estuaries (Glasby and Connell 1999), and their proximity to vessels. Vessel hulls are a major vector of introduction for sessile invertebrates (Gollasch 2002; Godwin 2003) and they present a similar habitat to pontoons as they float close to the water surface at a constant depth. Neves et al. (2007) found that species richness and composition were similar between floating pontoons and vessel hulls within the same marina, suggesting the potential for transfer of species between these structures. In contrast, pilings are fixed structures that maintain contact with the seafloor, experience a range of depths and have been found to support different assemblages to pontoons (Connell and Glasby 1999).

Past studies have investigated recruitment to experimental fixed and moving structures, but none has considered invader recruitment with respect to movement and depth, and variation in recruitment has rarely been examined over a small, shallow depth range. Perkol-Finkel et al. (2008) compared tropical assemblages between floating and fixed settlement plates and found compositional differences (at the level of functional group) relating to movement at depths of 1–12 m. Using a similar higher classification level, Holloway and Connell (2002) found swash and floatation contributed most to the development of a typical temperate pontoon fouling assemblage.

It has been hypothesised that NIS are most prevalent on floating structures like pontoons either because of proximity to the surface and/or because of movement (Glasby et al. 2007). This current study was designed to discriminate between these hypotheses with greater taxonomic resolution than used in previous studies, including classification of species as non-indigenous or native. The specific model tested was that the combined effects of movement and depth influence the richness of non-indigenous epifauna more than native epifauna. NIS were predicted to be most numerous on shallow, moving substrata (analogous to a floating pontoon) and more numerous than native epifauna. The depths chosen for the study were 0.5 and 2 m. The differential effects of moving and fixed structures on the recruitment and early development of fouling assemblages were tested while keeping constant other potentially confounding variables, for

example age and type of substratum. The results are interpreted with both reference to species that are non-indigenous to Australia and species that are native to Australia, but have been introduced overseas; the latter are termed 'exported' species (ES). Hereafter, NIS and ES are referred to as 'invaders'. For international relevance, it is important to consider invaders as a specific group including not only those species that pose a threat to Australia, but also species originating from Australia that might pose a threat to other countries.

## Methods

### Survey design

Experiments were conducted in Port Kembla, New South Wales, Australia, between October 2006 and December 2006 (Figure 1). Port Kembla is a major commercial harbour which receives approximately 600 local and international vessels a year (PKPC 2006). The harbour has a tidal range of 1.3 m and has a mixture of fixed and moving structures although the majority are fixed. To test for differences in the number of invaders on fixed or moving substrata, Perspex plates were attached to larger frames that were deployed vertically in the water column at two sites approximately 500 m apart. Moving frames had floats attached to allow them to remain at a constant depth below the surface despite changes in tides (similar to Holloway and Connell 2002; Figure 2a) and fixed frames were hung from jetties and weighted to remain stationary with the rise and fall of the tide (Figure 2b). All frames were deployed under the pier and experienced shaded conditions throughout the experiment. To test for differences in species richness and

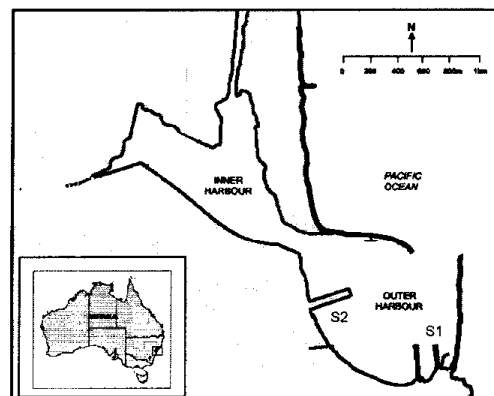


Figure 1. Locations of experimental sites in Port Kembla.

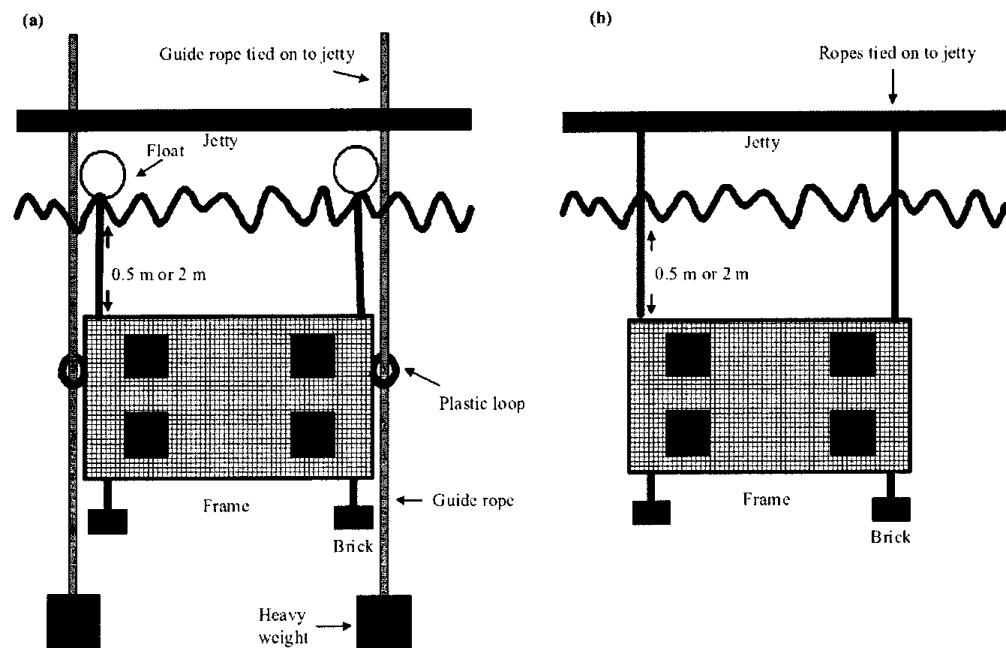


Figure 2. Diagram of the apparatus used to create (a) moving and (b) fixed structures. Moving frames were deployed at either 0.5 m or 2 m below the water surface. Fixed frames were at either 0.5 m or 2 m below MLWS (and their depth therefore varied according to the tides).

percentage cover as a result of depth, moving frames floated at 0.5 m or 2.0 m below the water surface, whereas fixed frames were fixed at 0.5 m or 2.0 m below mean low water springs (MLWS). Six (11 cm × 11 cm) replicate plates were deployed for each treatment combination (ie moving and shallow; moving and deep; fixed and shallow; fixed and deep).

To assess early fouling assemblage development, settlement plates were collected after 12 weeks when between 5 and 25% of bare space remained. Plates were photographed and preserved in 5% formalin before counting. The percentage cover of each species was estimated using a grid of 81 regularly spaced points placed over the preserved plate with a 1 cm border to account for edge effects. Photographs were used to assist in the identification of organisms on the plates. Organisms in the grid, but not under a point were noted and given a value of 0.5% cover. Organisms were identified to the lowest practical taxonomic level and classified as non-indigenous (NIS), exported (ES), native (N) or cryptogenic (C) (species without binomials, *sensu stricto* Carlton, 1996) according to the literature (Table 1). Cryptogenic species were included in the total species pool for

analyses. When considering invader richness, species non-indigenous to Australia and exported species that were invaders elsewhere were combined.

#### Data analysis

The frequency of occurrence of invaders vs. natives was compared for each treatment combination (ie moving and shallow; moving and deep; fixed and shallow; fixed and deep) using  $\chi^2$  tests. These tests related the frequency of occurrence to what would be expected by chance given the available species pool (number of NIS + ES + N + C). A three-factor ANOVA was used to compare the percentage cover of invader species between fixed and moving plates at different depths, and between sites. Movement and depth were treated as fixed orthogonal factors while site was considered random and orthogonal. All data were assessed for normality and homogeneity of variances using residual frequency histograms and Cochran's C test, respectively.  $\ln(x + 1)$  transformations were used to obtain homogeneous variances where necessary. SNK tests were used to identify significant differences between treatments.

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Table 1. List of species found during survey and their classification status as native (N), non-indigenous (NIS), exported (ES) and cryptogenic (C).

Phylum	Status (N/NIS/ES/C)	Native distribution	Invasive distribution	Source
Arthropoda				
<i>Amphibalanus variegatus</i>	ES	Indo-West Pacific (Including Australia and New Zealand)	Japan	Darwin (1854); Glasby et al. (2007); Otani (2007)
<i>Amphibalanus amphitrite</i>	NIS	Indo-West Pacific	Cosmopolitan	Darwin (1854); Ruiz et al. (2000); Hewitt et al. (2004)
<i>Balanus trigonus</i>	ES	Indo-West Pacific (Including Australia)	Hawaii	Darwin (1854); Ruiz et al. (2000); Glasby et al. (2007)
<i>Megabalanus coccopoma</i>	NIS	SE Pacific	NE Atlantic, S Atlantic, Australia	Darwin (1854); USGS (2008a)
<i>Austrobalanus imperator</i>	N	Australia		Darwin (1854); Glasby et al. (2007)
Cnidaria				
Hydroid 1	C			
Hydroid 2	C			
Ectoprocta				
<i>Arachnopusia unicornis</i>	N	Australia and New Zealand		Hutton (1873); Glasby et al. (2007)
<i>Beania magellanica</i>	N	Southern Ocean including Australia, New Zealand, South America, South Africa. (Cryptogenic in NW Pacific and NE Atlantic)		Busk (1852); Glasby et al. (2007)
<i>Bowerbankia gracilis</i>	NIS	W Atlantic	Cosmopolitan	Keough and Ross (1999); Ruiz et al. (2000);
<i>Bugula dentata</i>	ES	Indo-West Pacific including Australia (cryptogenic in South Africa)	Hawaii, S Pacific Islands	Lamoureux (1816); USGS (2008b)
<i>Bugula flabellata</i>	NIS	NE Atlantic	Cosmopolitan	Keough and Ross (1999); Hewitt et al. (2004)
<i>Bugula neritina</i>	NIS	NE Atlantic	Cosmopolitan	Keough & Ross (1999); Ruiz et al. (2000); Hewitt et al. (2004)
<i>Celleporaria nodulosa</i>	ES	Australia	New Zealand	Busk (1881); Aquenal Pty Ltd (2002); Inglis et al. (2006a,b)
<i>Conopeum seurati</i>	NIS	Mediterranean and NE Atlantic	Cosmopolitan	Keough & Ross (1999); Hewitt et al. (2004)
<i>Fenestrulina mutabilis</i>	N	Australia		Hastings (1932); Glasby et al. (2007)
<i>Microporella umbracula</i>	NIS	Mediterranean, W. Atlantic and Indo-Pacific	Australia?	Audouin (1826)
<i>Reteporella</i> sp.	C			
<i>Schizomavella</i> sp.	C			
<i>Schizoporella errata</i>	NIS	Mediterranean	Cosmopolitan	Keough & Ross (1999); Hewitt et al. (2004)
<i>Tricellaria inopinata</i>	NIS	NE and NW Pacific	Cosmopolitan	Keough & Ross (1999); Hewitt et al. (2004)
<i>Watersipora subtorquata</i>	NIS	E Pacific	Cosmopolitan	Ruiz et al. (2000)
Mollusca				
<i>Mytilus galloprovincialis planulatus</i>	N	Australia		Daguin (2000); Hilbish et al. (2000); Beu (2004)
Polychaeta				
<i>Galeolaria caespitosa</i>	N	Australia		Mörch (1863); Glasby et al. (2007)
<i>Hydroides elegans</i>	NIS	Unknown	Cosmopolitan	Haswell (1884); Hewitt et al. (2004)
<i>Hydroides diramphus</i>	NIS	Unknown	Australia	Mörch (1863); AMBS (2002)
<i>Pomatoceros taeniata</i>	N	Australia		Lamarck (1818); Aquenal Pty Ltd (2002, 2004)

(continued)

Table 1. (Continued).

Phylum	Status (N/NIS/ES/C)	Native distribution	Invasive distribution	Source
<i>Salmacina australis</i>	N	Australia		Haswell (1884); Aquenal Pty Ltd (2002, 2004)
Spirorbidae	C			
Porifera				
<i>Sycon</i> sp.	C			
Tunicata				
Ascidian I	C			
<i>Botrylloides magnicoecum</i>	N	Australia		Hartmeyer (1912); Pollard and Pethebridge (2002a,b)
<i>Botrylloides leachi</i>	NIS	NW Pacific	Cosmopolitan	Hewitt et al. (2004)
<i>Botryllus schlosseri</i>	NIS	NW Atlantic, NE Pacific, Australia, New Zealand	Cosmopolitan	Hewitt et al. (2004)
<i>Didemnum</i> spp.	C			
<i>Diplosoma listerianum</i>	NIS	NW Pacific	NW Atlantic, NE Atlantic, Hawaii, NE Pacific, Australia, New Zealand	Lambert & Lambert (1998); Ruiz et al. (2000)
<i>Herdmania momus</i>	ES	Australia	Hawaii	Savigny (1816); Pollard and Pethebridge (2002a,b); USGS (2008c)
<i>Styela plicata</i>	NIS	East Asian Seas	Caribbean, S Atlantic, NE Pacific, NW Pacific, Australia	Kott (1985); Keough and Ross (1999)

*D. listerianum* has been classified as NIS or C by different authors and in this study was treated as NIS following the reasoning of Ruiz et al. (2000).

In several analyses, movement and depth effects were variable between the sites. These small-scale differences have been interpreted in the text, but graphs have been presented for main effects only.

### Results

Thirty-nine taxa were identified during the study (most to genus or species). These included species of encrusting and arborescent bryozoans, barnacles, solitary and colonial ascidians and serpulid polychaetes. Sixteen species were identified as non-indigenous to Australia, six as exported, nine as native and eight as cryptogenic (Table 1). NIS alone were significantly more abundant than native species on all plates (Figure 3a; ANOVA:  $p < 0.01$ ). The frequency of occurrence of invaders and natives on shallow moving plates were significantly different from what would be expected by chance given the species pool (Figure 3a;  $\chi^2 = 5.23$ ,  $p < 0.05$ ). Thus, invaders occurred more frequently on shallow moving plates than would be expected by chance, and *vice versa* for native species. No significant pattern was detected on deep moving plates ( $\chi^2 = 3.11$ ,  $p > 0.05$ ), shallow fixed plates ( $\chi^2 = 2.36$ ,  $p > 0.05$ ) or deep fixed plates ( $\chi^2 = 2.52$ ,  $p > 0.05$ ).

### Effects of movement on invader recruitment

Several species showed a positive response to movement. The non-indigenous ascidian *Diplosoma listerianum* (Milne-Edwards 1841) and the exported ascidian, *Herdmania momus* (Savigny 1816) both had significantly greater percentage cover on moving compared to fixed plates (Figure 3b and 3c; Table 2(a) and (b)). The non-indigenous ascidian *Botrylloides leachi* (Savigny 1816), non-indigenous barnacle *Megabalanus coccopoma* (Darwin 1854) and exported barnacle *Amphibalanus variegatus* (= *Balanus variegatus*) Darwin 1854) also occupied more space on moving plates (Figure 3d–3f; Table 2(c)–(e)), but this was only significant at one site. In contrast, the exported barnacle *Balanus trigonus* (Darwin 1854) and the non-indigenous bryozoan *Microporella umbracula* (Audouin 1826) occupied significantly more space on fixed plates (Figure 3g and 3h; Table 2(f) and (g)). The cover of the non-indigenous ascidian *Styela plicata* (Lesueur 1823) and bryozoan *Conopeum seurati* (Canu 1928) was also greater on fixed plates (Figure 3(i) and 3(j); Table 2(h) and (i)), but this was only significant at one site.

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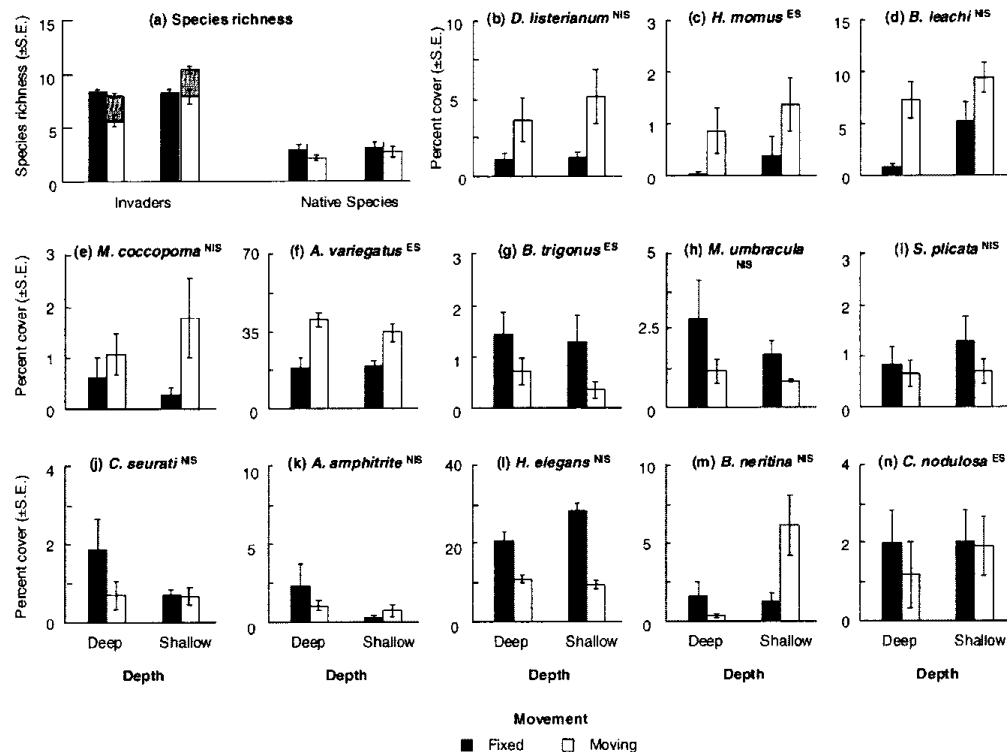


Figure 3. (a) Species richness of invaders (NIS = filled; ES = hatched) and native species; (b)–(n) percentage cover of non-indigenous species (NIS) and exported species (ES) on fouling plates attached to fixed or moving frames and deployed at 0.5 or 2 m depth. Data were pooled for sites.

#### Effect of depth on invader recruitment

The non-indigenous barnacle *Amphibalanus amphitrite* (= *Balanus amphitrite*) Darwin 1854; see Evans 2009) was the only species to show an overall effect of depth and occupied more space on deep than shallow plates (Figure 1k; Table 2(j)). The percentage cover of *H. momus*, *B. leachi* and *S. plicata* also varied with depth and were greater on shallow than deep frames (Figure 3c, 3d, 3i; Table 2(b), (c), (h)), but this was only significant at one site. In contrast, *C. seurati* occupied more space on deep than shallow frames (Figure 3j; Table 2(i)) although this again was only significant at one site.

#### Interactive effect of movement and depth on invader recruitment

Cover of the non-indigenous serpulid *Hydroides elegans* (Haswell 1884) varied according to movement, depth and site (Figure 3l; Table 2(k)). The serpulid

occupied almost twice as much space on fixed plates compared to moving plates regardless of depth and, at one site, had a greater cover on shallow fixed plates than on deep fixed plates. Cover of the non-indigenous bryozoan *Bugula neritina* (Linnaeus 1758) also showed an interaction between movement, depth and site (Figure 3m; Table 2(l)). *B. neritina* occupied by far the most space on moving plates at shallow depths, but at one site, occupied more space on fixed than on moving plates (Figure 3m; Table 2(l)). Cover of the exported bryozoan *Celleporaria nodulosa* (Busk 1881) differed between sites, but showed no effect of movement or depth (Figure 3n, Table 2(m)).

#### Native species recruitment

Only four native species occupied > 1% space, viz. the mussel *Mytilus galloprovincialis planulatus* (Lamarck 1819), the serpulid *Galeolaria caespitosa* (Mörch 1863), the bryozoan *Fenestrulina mutabilis* (Hastings 1932) and the ascidian *Botrylloides magnicoecum*

Table 2. Analyses of variance comparing percentage cover of non-indigenous (NIS) and exported species (ES) on fouling plates that were attached to fixed or moving frames at different depths.

Source	df	MS	F	P	MS	F	P	MS	F	P	MS	F	P
(a) <i>D. listerianum</i> <sup>NIS</sup> ln(x + 1)													
Movement	1	5.997	11.01	<b>0.002</b>	2.099	9.02	<b>0.005</b>	341.646	2.26	0.374	2.107	1.63	0.423
Depth	1	0.433	0.79	0.378	0.354	0.21	0.729	129.338	0.90	0.517	0.013	0.05	0.832
Site	1	1.089	2.00	0.165	0.665	2.86	0.099	1.864	0.10	0.753	0.130	0.46	0.499
Mo x De	1	0.002	0.00	0.957	0.013	0.02	0.913	16.229	0.87	0.356	0.357	1.28	0.265
Mo x Si	1	0.226	0.42	0.523	0.007	0.03	0.867	151.072	8.12	<b>0.007</b>	1.292	4.62	<b>0.037</b>
De x Si	1	0.220	0.40	0.529	1.718	7.38	<b>0.010</b>	144.076	7.74	<b>0.008</b>	0.326	1.17	0.286
Mo x De x Si	1	0.068	0.12	0.726	0.688	2.96	0.093	19.055	0.556	0.864	0.034	0.12	0.730
Error	40	0.573			0.238						0.285		
(b) <i>H. momus</i> <sup>ES</sup> ln(x + 1)													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561
Error	40	0.274			0.496			0.209			0.170		
(c) <i>B. leachi</i> <sup>NIS</sup>													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561
Error	40	0.274			0.496			0.209			0.170		
(d) <i>M. coccopoma</i> <sup>NIS</sup> ln(x + 1)													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561
Error	40	0.274			0.496			0.209			0.170		
(e) <i>A. variegatus</i> <sup>ES</sup>													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561
Error	40	0.274			0.496			0.209			0.170		
(f) <i>B. trigonus</i> <sup>ES</sup> ln(x + 1)													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561
Error	40	0.274			0.496			0.209			0.170		
(g) <i>M. umbracula</i> <sup>NIS</sup> ln(x + 1)													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561
Error	40	0.274			0.496			0.209			0.170		
(h) <i>S. plicata</i> <sup>NIS</sup> ln(x + 1)													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561
Error	40	0.274			0.496			0.209			0.170		
(i) <i>C. seurati</i> <sup>NIS</sup> ln(x + 1)													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561
Error	40	0.274			0.496			0.209			0.170		
(j) <i>A. amphitrite</i> <sup>NIS</sup> ln(x + 1)													
Movement	1	1.522	5.90	<b>0.019</b>	2.268	4.70	<b>0.036</b>	0.207	0.19	0.741	0.664	0.53	0.600
Depth	1	0.281	1.09	0.303	0.181	0.38	0.543	0.164	0.16	0.756	0.196	0.10	0.809
Site	1	0.237	0.92	0.343	0.028	0.06	0.810	2.544	12.17	<b>0.001</b>	1.335	8.00	<b>0.007</b>
Mo x De	1	0.018	0.07	0.791	0.008	0.01	0.949	0.070	0.21	0.729	0.445	2.66	0.110
Mo x Si	1	0.121	0.47	0.497	0.125	0.26	0.614	1.119	5.35	<b>0.026</b>	1.258	7.53	<b>0.009</b>
De x Si	1	0.002	0.01	0.924	0.300	0.62	0.435	1.003	4.80	<b>0.034</b>	2.043	12.24	<b>0.001</b>
Mo x De x Si	1	0.003	0.01	0.912	1.263	2.62	0.113	0.341	1.63	0.209	0.058	0.34	0.561



(Hartmeyer 1912). *M. galloprovincialis planulatus* was the dominant native space occupier and its cover varied significantly with respect to movement, depth and site (ANOVA;  $F_{1,40} = 5.3$ ,  $p = 0.0266$ ). The mussel was more abundant on shallow moving plates at one site and deep moving plates at the other. In contrast, *G. caespitosa* and *F. mutabilis* occupied more space on fixed than moving plates, but this was significant only at one site (ANOVA;  $F_{1,40} = 6.15$ ,  $p = 0.0172$  and  $F_{1,40} = 5.47$ ,  $p = 0.0242$ , respectively). *B. magnicoecum* showed no effect of movement or depth.

### Discussion

This study has demonstrated the combined influences of depth and movement on the early development of marine epifaunal assemblages. As predicted, marine invaders (NIS and ES) were disproportionately more abundant on shallow (0.5 m) moving surfaces than on deep (2 m) moving or fixed surfaces. Moreover, NIS alone were more species rich on all plates, at both depths, than native species. Effects of movement and/or depth were detected for some individual species and in many cases these effects were site-specific, and/or occurred for only fixed or only moving plates.

Various studies have documented differences in recruitment of epifauna at depths much greater than 2 m (eg Aleem 1957; Jackson and Winston 1982; Hughes and Jackson 1992), but few have examined depth-related differences in surface waters. Certainly it has been known for a long time that boat hulls tend to be fouled more heavily in the first meter or so of water, but fouling can extend to a depth of a few metres (Visscher 1927). McDougall (1943) noted the luxuriant growth of the invasive bryozoan *Bugula neritina* on floats 15 cm–1 m deep, and a marked decline in its abundance at greater depths. Similarly, Hurlbut (1991b) found that a colonial ascidian was more abundant 15 cm below the surface on a float than at depths of 1–6 m on a fixed piling. But why are many invasive epifauna more prevalent on floating surfaces than just 1.5 m deeper? The most obvious differences between floating structures at the surface vs. those 2 m deep are levels of light, turbulence (eg due to swash; Holloway and Connell 2002), temperature and salinity. Freshwater layers can persist for days on the surface after heavy rainfall (T.M. Glasby, personal observations) and different marine fouling species have varying susceptibility to freshwater (Visscher 1927). All these factors are likely to be more variable and extreme at 0.5 m than at 2 m (eg increased turbulence around floating docks, Koehl 2007). Thus, the greater proportion of invasive epifauna on shallow moving surfaces might relate to their enhanced ability to withstand

physical disturbances, as predicted by the disturbance-invasibility hypothesis (Elton 1958). The potential influences of these physical variables on epifauna are discussed below.

Several species showed differences in their space occupation with depth, independent of movement. Previous work on pontoons and pilings has highlighted the effect of light availability on these structures as a factor influencing the recruitment of larvae to their surfaces (Glasby 1999b). Light can be expected to decrease with depth and may influence the growth of various algal species; a semi-shaded substratum will favour the competitive exclusion of algae by sessile invertebrates (Glasby 1999b). Differences in recruitment between shallow and deep plates may have been influenced by light availability, particularly larval responses to light. Colonial ascidians have been found to recruit in largest numbers on floating docks, and this is thought to be a result of their photopositive behaviour on release which results in them swimming upwards (Hurlbut 1991a). Similar to the colonial ascidians, the invasive bryozoan *B. neritina* is photosensitive during the larval stage, although it becomes indifferent to light prior to metamorphosis (Lynch 1947). In this study, *B. neritina* occupied more space on moving plates at shallow depths (although this was only significant at one site).

Several non-indigenous or exported species occupied more space on moving than on fixed plates at both depths. Their increased percentage cover on moving substrata could be in response to stronger water flow over moving surfaces (Perkol-Finkel et al. 2006). Numerous studies have demonstrated that increased water flow can result in increased recruitment of epifauna (eg Mullineaux and Garland 1993; Judge and Craig 1997; Leonard et al. 1998). Increased water flow also has the potential to increase food availability and therefore increase size (and aeral coverage) of epifauna (Eckman and Duggins 1993; Perkol-Finkel et al. 2008). In the case of filter-feeding invaders, the ability to settle and remain attached under high shear stresses could advantage them during settlement and transport on boat hulls (see review by Koehl 2007), and may have been an important factor in facilitating the spread of invaders on the hulls of ships and their subsequent ability to establish and persist, especially on floating surfaces in ports and estuaries around the world.

In contrast, some invaders including the non-indigenous serpulid *H. elegans* occupied more space on fixed than moving structures, although this did vary between sites. The percentage cover of *H. elegans* may have been greater on fixed structures due to the preference of serpulid larvae to settle in areas with reduced water flow. Glasby (2001) found that the cover

of *H. elegans* decreased with increasing water flow and Mullineaux and Garland (1993) found that the closely related *H. dianthus* avoided settling in areas with considerable water flow.

Although invaders as a group were found to be more abundant on shallow moving surfaces, it is important to acknowledge the variability in the magnitude of the effect of movement and depth between sites. Some species displayed effects of movement and/or depth that were consistent across sites. However, several species showed localised effects. This variability may be related to the duration of the study and/or to the spatial scale. The recruitment of sessile organisms can vary considerable over small spatial and temporal scales (Sutherland and Karlson 1977; Keough and Butler 1983). Thus, it will be important to repeat the experiment described here in a variety of different locations to test for the generality of these results. The identity of species in any fouling assemblage will differ among locations and times, but the main question to answer is whether invasive epifauna in general, or particular wide-spread or problematic invaders, are consistently most abundant on shallow moving surfaces and more abundant than native epifauna. Arguably a study of longer duration might have lead to the results being more spatially consistent, but Butler and Connolly (1999) found overall assemblage composition on pier pilings had not converged to a 'stable' state even after 6.5 years.

Notably, some invaders showed opposite responses to fixed and moving treatments including the exported barnacle *B. trigonus* and non-indigenous bryozoan *M. umbracula* (more on fixed) compared to the non-indigenous ascidian *D. listerianum* and exported ascidian *H. momus* (more on moving). Native species as a group showed no effect of movement or depth, however, individually they showed varying responses to fixed and moving plates. The dominant space occupier *M. galloprovincialis planulatus* occupied more space on moving plates and in most cases this was regardless of depth. For the purposes of this study *M. galloprovincialis planulatus* has been classified as native (according to Daguin and Borsa 2000; Hilbish et al. 2000; Beu 2004). However, this genus is currently under genetic review and it is possible that the species is exported. Its abundance on moving structures would make it a likely candidate for transport on vessel hulls and establishment on pontoons in other countries.

A good understanding of the ecological effects of adding artificial structures to the coastal environment is essential for informing management strategies. The construction of boating structures creates novel habitat and epibiotic assemblages have been shown to develop differently on these structures compared to nearby

natural rocky reef (Connell 2001; Knott et al. 2004; Glasby et al. 2007). This study has demonstrated the potential for shallow moving surfaces to promote invader dominance and goes some way to explaining why moving structures such as vessel hulls (particularly sections close to the water surface), pontoons and navigation markers are 'hotspots' for invaders. This adds to a growing body of findings related to invisibility, including that invaders are more abundant on concrete surfaces (Glasby et al. 2007), on copper treated surfaces and in areas with high levels of copper contamination (Piola and Johnston 2007, 2008; Dafforn et al. 2008, personal communication). Future research should consider multifactorial experiments to tease apart the relative contributions of all these factors in enhancing invasibility.

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Appendix V: Full ANOVA tables for Chapter 4

Analyses of variance comparing percent covers of native species on fouling plates that were attached to fixed or moving frames at different depths.

% cover	<i>Mytilus galloprovincialis planulatus</i>				<i>Galeolaria caespitosa</i>				<i>Fenestrulina nutabilis</i>				<i>Botrylloides magnicoecum</i>			
Source	DF	MS	F	P	MS	F	P	P	MS	F	P	P	MS	F	P	P
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Movement	1	96.05	2.33	0.369	7.69	2.02	0.391		157.82	1.80	0.408		0.01	0.01		0.928
Depth	1	0.03	0.01	0.955	0.49	0.79	0.379		22.30	1.39	0.244		0.50	1.41		0.242
Site	1	26.70	10.11	0.003	3.07	4.94	<b>0.032</b>		47.07	2.94	0.094		3.34	9.42		<b>0.004</b>
Mo x De	1	0.13	0.01	0.940	0.55	0.89	0.350		47.07	2.94	0.094		0.12	0.35		0.557
Mo x Si	1	41.15	15.58	<b>0.000</b>	3.81	6.15	<b>0.017</b>		87.52	5.47	<b>0.024</b>		1.14	3.21		0.080
De x Si	1	6.22	2.36	0.133	0.00	0.00	0.986		19.06	1.19	0.281		0.48	1.34		0.253
Mo x De x Si	1	14.00	5.30	<b>0.027</b>	0.01	0.01	0.924		1.34	0.08	0.774		0.14	0.39		0.537
Error	40	2.64			0.65				16.28				0.36			

**Bold** indicates significantly different at  $P < 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .

Appendix VI: Full ANOVA tables for Chapter 5

Analyses of variance comparing percent covers of non-indigenous species on pilings, pontoons and rocky reefs at four sites in Port Jackson in 2006 and 2007.

% cover	Cryptosula pallasiana					Botrylloides leachi					Watersipora subtorquata				
	Source	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P	
Structure		2	1419.61	0.80	0.491	1500.39	2.08	0.206	168.09	0.48	0.643				
Site		3	2157.26	46.50	<b>0.000</b>	558.73	18.07	<b>0.000</b>	305.20	13.17	<b>0.000</b>				
Time		1	2543.73	1.33	0.332	266.47	0.66	0.475	9.01	0.04	0.859				
St x Si		6	1770.00	38.16	<b>0.000</b>	722.18	23.36	<b>0.000</b>	353.62	15.26	<b>0.000</b>				
St x Ti		2	1228.37	0.92	0.448	68.00	0.22	0.808	38.94	0.29	0.759				
Si x Ti		3	1905.47	41.08	<b>0.000</b>	402.17	13.01	<b>0.000</b>	242.13	10.45	<b>0.000</b>				
St x Si x Ti		6	1334.81	28.77	<b>0.000</b>	307.10	9.93	<b>0.000</b>	135.00	5.82	<b>0.000</b>				
Error		336	46.39			30.92			23.18						

**Bold** = significantly different at  $\alpha = 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .

Analyses of variance comparing percent covers of non-indigenous species on pilings, pontoons and rocky reefs at four sites in Port Jackson in 2006 and 2007.

% cover	Tricellaria inopinata				Styela plicata				Dictyota dichotoma			
	Source	DF	MS	F	P	MS	F	P	MS	F	P	
Structure		2	69.65	2.38	0.174	154.43	3.33	0.106	513.03	2.82	0.137	
Site		3	26.48	7.25	<b>0.000</b>	31.13	6.47	<b>0.000</b>	847.37	28.23	<b>0.000</b>	
Time		1	30.95	1.94	0.258	0.43	0.01	0.929	359.44	16.28	0.027	
St x Si		6	29.31	8.02	<b>0.000</b>	46.37	9.64	<b>0.000</b>	181.89	6.06	<b>0.000</b>	
St x Ti		2	33.51	2.41	0.170	1.46	0.03	0.969	56.30	0.18	0.840	
Si x Ti		3	15.95	4.36	<b>0.005</b>	46.79	9.73	<b>0.000</b>	22.08	0.74	0.531	
St x Si x Ti		6	13.89	3.80	<b>0.001</b>	45.54	9.47	<b>0.000</b>	313.24	10.44	<b>0.000</b>	
Error		336	3.65			4.81			30.01			

**Bold** indicates significantly different at  $p < 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .

Analyses of variance comparing percent covers of non-indigenous and native species (invertebrates and algae) in new and established assemblages on sandstone plates deployed vertically or horizontally and subjected to three shading treatments at two sites in Port Jackson.

% cover	Non-indigenous species				Native species				Native invertebrates				Native algae			
	DF	MS	F	P	MS	F	P	MS	MS	F	P	MS	MS	F	P	P
Assemblage	1	14.1601	43.37	<b>0.000</b>	21.109	6.74	0.234	0.2554	0.04	0.868		2774.408	45.99	<b>0.000</b>		
Orientation	1	267.366	7.17	0.228	188.199	1.99	0.393	711.512	3.38	0.317		1074.008	17.8	<b>0.000</b>		
Shading	2	0.3192	0.98	0.380	0.3565	0.84	0.437	1.7017	2.5	0.087		962.2583	15.95	<b>0.000</b>		
Site	1	46.9744	143.87	<b>0.000</b>	41.1574	96.48	<b>0.000</b>	76.1295	111.71	<b>0.000</b>		12834.01	212.73	<b>0.000</b>		
As x Or	1	4.5189	13.84	<b>0.000</b>	1.2964	0.71	0.555	5.9836	6.08	0.245		226.875	1.19	0.472		
As x Sh	2	0.0917	0.28	0.756	1.3805	3.24	<b>0.043</b>	0.2675	0.39	0.676		5.2083	0.09	0.917		
As x Si	1	0.1231	0.38	0.541	3.1309	7.34	<b>0.008</b>	5.8079	8.52	<b>0.004</b>		21.675	0.36	0.550		
Or x Sh	2	0.0959	0.29	0.746	6.0968	14.29	<b>0.000</b>	1.781	1.81	0.356		170.8083	2.83	0.063		
Or x Si	1	37.2966	114.23	<b>0.000</b>	94.6363	221.84	<b>0.000</b>	210.545	308.94	<b>0.000</b>		2.4083	0.04	0.842		
Sh x Si	2	0.0293	0.09	0.914	0.2528	0.59	0.555	0.3998	0.59	0.558		22.9083	0.38	0.685		
As x Or x Sh	2	0.0134	0.04	0.960	0.2402	0.56	0.571	0.2768	0.41	0.667		74.425	1.23	0.295		
As x Or x Si	1	0.3621	1.11	0.295	1.8356	4.3	<b>0.041</b>	0.9842	1.44	0.232		190.0083	3.15	0.079		
As x Sh x Si	2	0.4404	1.35	0.264	0.3322	0.78	0.462	0.0942	0.14	0.871		37.525	0.62	0.539		
Or x Sh x Si	2	0.0957	0.29	0.747	0.5079	1.19	0.308	0.9852	1.45	0.240		76.4583	1.27	0.286		
As x Or x Sh x Si	2	0.2634	0.81	0.449	0.5976	1.4	0.251	0.5189	0.76	0.470		55.8083	0.93	0.400		
Error	96	0.3382			0.4269			0.703				62.35				

**Bold** indicates significantly different at  $P < 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .



Reduced model analyses of variance comparing percent covers of non-indigenous species in new and established assemblages on sandstone plates subjected to three shading treatments at two sites in Port Jackson.

% cover	<i>Diplosoma listerianum</i>				<i>Watersipora subtorquata</i>				<i>Cryptosula pallasiana</i>			
	Source	DF	MS	F	P	MS	F	P	MS	F	P	P
Assemblage		1	0.2093	0.63	0.432	1.8547	2.89	0.339	0.5918	0.77	0.541	
	Shading	2	0.1961	0.59	0.560	0.0964	0.42	0.657	0.1106	0.63	0.537	
Site		1	0.4596	1.38	0.246	1.6469	7.22	<b>0.010</b>	5.625	32.01	<b>0.000</b>	
	Asx Sh	2	1.0001	1.68	0.373	0.1057	0.46	0.632	0.0569	0.32	0.725	
As x Si		1	0.0137	0.04	0.840	0.6419	2.82	0.099	0.7678	4.37	<b>0.042</b>	
	Sh x Si	2	0.0173	0.05	0.950	0.2204	0.97	0.387	0.1658	0.94	0.396	
As x Sh x Si		2	0.5959	1.78	0.178	0.2333	1.02	0.367	0.0187	0.11	0.899	
	Error	48	0.3541			0.2281			0.1827			

**Bold** indicates significantly different at  $P < 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .

Analyses of variance comparing percent covers of non-indigenous species in new and established assemblages on sandstone plates deployed vertically or horizontally and subjected to three shading treatments at two sites in Port Jackson.

% cover	Watersipora arcuata					Botrylloides leachi			Hydroides elegans			Schizoporella errata		
Source	DF	MS	F	P		MS	F	P	MS	F	P	MS	F	P
Assemblage	1	0.0149	0.32	0.573		0.1837	1.34	0.454	8.5012	27.14	<b>0.000</b>	12.6491	2.74	0.346
Orientation	1	0.5326	1.18	0.474		1.2219	1.52	0.434	79.8289	4.59	0.278	53.5963	5.49	0.257
Shading	2	0.0493	1.06	0.352		0.0475	0.85	0.430	0.0731	0.23	0.792	0.3444	1.16	0.318
Site	1	0.4513	9.66	<b>0.002</b>		0.7718	13.83	<b>0.000</b>	22.1386	70.69	<b>0.000</b>	10.1289	34.07	<b>0.000</b>
As x Or	1	0.0008	0.02	0.896		0.087	1.56	0.215	1.02	3.26	0.074	11.2042	2.56	0.356
As x Sh	2	0.0738	0.83	0.548		0.0968	1.19	0.457	0.0155	0.05	0.952	0.6626	2.23	0.113
As x Si	1	0.0076	0.16	0.688		0.1374	2.46	0.120	0.0384	0.12	0.727	4.6196	15.54	<b>0.000</b>
Or x Sh	2	0.053	1.13	0.325		0.0205	0.37	0.693	0.3652	1.17	0.316	0.1521	0.51	0.601
Or x Si	1	0.4513	9.66	<b>0.002</b>		0.803	14.39	<b>0.000</b>	17.3987	55.55	<b>0.000</b>	9.7629	32.84	<b>0.000</b>
Sh x Si	2	0.01	0.21	0.808		0.0732	1.31	0.274	0.0799	0.26	0.775	0.0062	0.02	0.979
As x Or x Sh	2	0.0399	0.85	0.429		0.0799	1.43	0.244	0.1846	0.59	0.557	0.3714	1.25	0.291
As x Or x Si	1	0.0076	0.16	0.688		0.0561	1.01	0.318	0.3348	1.07	0.304	4.3735	14.71	<b>0.000</b>
As x Sh x Si	2	0.0893	1.91	0.153		0.0815	1.46	0.237	0.21	0.67	0.514	0.3539	1.19	0.308
Or x Sh x Si	2	0.0377	0.81	0.449		0.0662	1.19	0.310	0.0449	0.14	0.867	0.0043	0.01	0.986
As x Or x Sh x Si	2	0.0398	0.85	0.429		0.0498	0.89	0.413	0.2703	0.86	0.425	0.2972	1	0.372
Error	96	0.0486				0.0554			0.3294			0.3083		

**Bold** indicates significantly different at  $P < 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .

Analyses of variance comparing light intensity on sandstone plates subjected to three shading treatments at two sites in Port Jackson.

<b>Light intensity</b>				
<b>Source</b>	<b>DF</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Shading	2	120.23	30.21	<b>0.032</b>
Site	1	58.35	66.15	<b>0.000</b>
Sh x Si	2	3.98	4.51	<b>0.012</b>
Error	330	0.88		

Analyses of variance comparing sediment loads collected from new and established assemblages on sandstone plates deployed vertically or horizontally and subjected to three shading treatments at two sites in Port Jackson.

<b>Sediment (plates)</b>				
<b>Source</b>	<b>DF</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Assemblage	1	2.36	25.78	<b>0.000</b>
Orientation	1	0.10	1.09	0.303
Shading	2	2.88	5.96	0.248
Site	1	0.12	1.26	0.297
As x Or	1	0.03	0.34	0.561
As x Sh	2	0.48	5.26	<b>0.028</b>
As x Si	1	0.07	0.81	0.452
Or x Sh	2	0.18	2.01	0.165
Or x Si	1	0.05	0.49	0.616
Sh x Si	2	0.16	1.74	0.191
As x Or x Sh	2	0.08	0.90	0.351
As x Or x Si	1	0.07	0.82	0.451
As x Sh x Si	2	0.08	0.84	0.442
Or x Sh x Si	2	0.07	0.78	0.467
As x Or x Sh x Si	2	0.13	1.37	0.268
Error	96	0.10		

**Bold** indicates significantly different at  $P < 0.05$ . *Post-hoc* pooling done at  $P > 0.25$ .

**Appendix VII:** Piola, R. F., K. A. Dafforn, and E. L. Johnston. 2009. The influence of antifouling practices on marine invasions: a mini-review. *Biofouling* **25**:633-644

## MINI REVIEW

### The influence of antifouling practices on marine invasions

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Vessel hull-fouling is increasingly recognised as one of the major vectors for the transfer of marine non-indigenous species. For hundreds of years, copper (Cu) has been used as a primary biocide to prevent the establishment of fouling assemblages on ships' hulls. Some non-indigenous fouling taxa continue to be transferred *via* hull-fouling despite the presence of Cu antifouling biocides. In addition, several of these species appear to enjoy a competitive advantage over similar native taxa within metal-polluted environments. This metal tolerance may further assist their establishment and spread in new habitats. This review synthesises existing research on the links between Cu and the invasion of fouling species, and shows that, with respect to the vector of hull-fouling, tolerance to Cu has the potential to play a role in the transfer of non-indigenous fouling organisms. Also highlighted are the future directions for research into this important nexus between industry, ecology and environmental management.

**Keywords:** copper (Cu); antifouling; non-indigenous species (NIS); hull-fouling; pollution; tolerance; invasion

#### Introduction

The introduction and establishment of non-indigenous species (NIS) can have profound effects on the economic potential, social values and environmental stability of affected regions (Vitousek et al. 1997; Mack et al. 2000; Pimentel et al. 2005; Colautti et al. 2006). Within the marine environment, ports and harbours are the primary 'hot-spots' for the increased occurrence and abundance of non-indigenous marine species (Cohen and Carlton 1998; Minchin and Gollasch 2003; Drake and Lodge 2004), with international shipping being one of the most important vectors responsible for their spread between regions (Otani et al. 2007; Hewitt et al. 2009; Yamaguchi et al. 2009). Furthermore, ports can act as stepping-stones for the intra-regional spread of unwanted species, *via* human-mediated pathways (eg domestic vessel traffic) or natural dispersal (Floerl et al. 2009b; Forrest et al. 2009).

Proliferation of NIS in ports and harbours occurs despite the fact these environments receive high levels of anthropogenic disturbance, such as chemical pollution (Hall Jr et al. 1998; Kennish 2002). Certain common marine pollutants such as copper (Cu) and zinc (Zn) are intrinsically associated with some modes of NIS transfer (eg vessel hull fouling) through their use as primary biocides in antifouling (AF) coatings. While the effects of toxicants in bays and estuaries have been of concern for a long time and have been

discussed extensively (Hartman 1960; Phillips 1977; Preston and Shackelford 2002), concerted attention to the establishment and spread of invasive species in marine systems has occurred more recently (Carlton and Geller 1993; Ruiz et al. 2000). There is comparatively little understanding of the interactive and/or cumulative effects of pollution and invasion, especially given their regular co-occurrence at both the transport- and establishment-stages of the marine invasion process. As an ever increasing global population places more stress on coastal environments (eg poor water quality, habitat loss, decreased biodiversity, nutrient enrichment; Kennish 2002; Preston and Shackelford 2002; Goldman and Wasson 2008), and the reliance on shipping for the transport of goods and services worldwide increases, it becomes important to understand the links between toxicants and invasions in order to maintain the integrity of near-shore marine environments.

The application of AF coatings is a necessary and important maintenance requirement for all vessels. From an economic perspective, it is now recognised that even minor fouling, such as slime-film layers, can have significant impacts on the operating efficiency of affected vessels, resulting in increased fuel requirements to maintain desired operating speeds (Schultz 2007). From an environmental standpoint, AF biocides are vital to minimising the global spread of unwanted organisms *via* international vessel traffic

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(Evans et al. 2000). In spite of their importance and necessity, all biocidal AF paints are heavily scrutinised by regulatory authorities, due to environmental concerns arising from heavy metal pollution (Valkirs et al. 2003; Srinivasan and Swain 2007), bioaccumulation in marine organisms (Claisse and Alzieu 1993) and adverse effects on human health (Evans et al. 2000). These concerns have led to increased research into the development of non-biocidal coatings, such as silicone-based, fouling-release technology (eg Brady 2001; Candries et al. 2001; Kavanagh et al. 2005; Kim et al. 2007). However, as fouling-release coatings do not prevent the accumulation of fouling, but rather 'release' it as the vessel moves through the water, the potential for transmission of NIS appears to be high. As a result, AF formulations containing biocides such as Cu continue to be the most popular (and arguably most effective) broad-spectrum AF treatments in the market today (Srinivasan and Swain 2007).

AF biocides, such as Cu, exert strong selective pressures on both the target pests and non-target organisms, favouring individuals with increased resistance (Russell and Morris 1970, 1972; Reed and Moffat 1983; Floerl et al. 2004; Han et al. 2008; Piola and Johnston 2008a, Forthcoming 2009). As such, the use of Cu-based antifoulants on vessel hulls has the potential to select for the settlement and transport of non-indigenous organisms with a high tolerance to metal pollution. If these tolerant individuals are then transported to polluted recipient locations, a growing body of evidence suggests they may benefit from some degree of competitive advantage over native species (eg Dafforn et al. 2008; Han et al. 2008; Piola and Johnston 2008a; Dafforn et al. Forthcoming 2009; Piola and Johnston, Forthcoming 2009). Therefore, the question must be asked: could the presence of metal pollution at the transport- and establishment-stage of the invasion process facilitate the introduction and spread of NIS?

#### History of Cu as an AF agent

The toxic nature of Cu has been studied in detail. Early life history stages of marine invertebrates and algae are negatively affected by Cu concentrations in the 5–100  $\mu\text{g l}^{-1}$  range, which is at least an order of magnitude lower than concentrations that may be toxic to humans (Hall Jr et al. 1998; Spencer 2003). Hence, Cu, in numerous forms, has a very long history of use as an antifoulant, and is still one of the most effective and practical means of preventing fouling on submerged aquatic structures. The first successful AF surface to receive widespread recognition was Cu sheathing, with the ancient Phoenicians and Carthaginians being credited with the first documented

use of such sheathing on vessel hulls c. 700 BC (Almeida et al. 2007), though it did not become common practice until the 18th century (WHOI 1952). In the mid 1800s, the first widespread general-use AF coating (named 'McInness') was introduced in the Liverpool dockyards (in the UK), and used Cu sulphate as the toxicant (Yebra et al. 2004). A century later, the broad spectrum AF effectiveness of tributyltin (TBT) compounds became recognised (Huggett et al. 1992; Evans et al. 2000). TBT could be incorporated into a highly effective Self-Polishing Copolymer (SPC) paint matrix and as a result, TBT SPC coatings became the new standard for vessel AF for years to come (Yebra et al. 2004; Almeida et al. 2007). Towards the end of the 1970s however, the environmental impacts of TBT antifoulants raised concern, with links established between TBT and deformities in shellfish (Scammell et al. 1991), imposex in gastropods (Foale 1993; Wilson et al. 1993; Andersen 2004) and bioaccumulation in tissues of marine vertebrates (Harino et al. 2000). By the 1980s, many countries had banned the use of TBT paints on vessels <25 m in length (Evans et al. 2000), and by 2008 the International Maritime Organisation (IMO) had expanded this ban to include all non-government and non-navy vessels (IMO 2001; Lewis et al. 2004). As a result, Cu-based AF paints regained popularity, and are likely to remain the dominant AF method until more advanced technological solutions become widely available (eg Depree 2009). There exist several comprehensive reviews which discuss the range of AF technologies (past and present) used on maritime vessels (Yebra et al. 2004; Almeida et al. 2007).

Whilst TBT is not 100 percent effective at preventing all fouling growth on vessel hulls (Rainer 1995; Gollasch 2002; Minchin and Gollasch 2003), Cu is generally considered to be a less effective AF biocide against a broad range of taxa when compared to TBT. This can be attributed to both its reduced toxicity compared to organotin (Raikina 2004), and the fact that most Cu-based paints have reduced life-spans, and are less efficient and cost-effective compared to TBT-based self-polishing paints (Yebra et al. 2004; Almeida et al. 2007). From the perspective of marine invasions, this will invariably impact upon the numbers and types of organisms able to be transferred globally by fouling-related vectors such as vessel hull-fouling. Several groups of sessile marine organisms have shown significant tolerance to Cu, including calcareous tube-worms (Johnston and Keough 2003; Dafforn et al. 2008) barnacles (Weiss 1947), hydroids (Stebbing 2002), bryozoans (Floerl et al. 2004; Piola and Johnston 2006a), bivalves (Lee and Chown 2007) and algae (Russell and Morris 1970, 1972; Reed and Moffat 1983; Correa et al. 1996; Jelic-Mrcelic et al.

2006; Han et al. 2008). All these groups of taxa have similarly been associated with regional introductions and spread *via* vessel hull-fouling. A growing body of evidence suggests that these two factors may be related (eg Dafforn et al. 2008; Piola and Johnston 2008a, Forthcoming 2009; Dafforn et al. Forthcoming 2009).

#### Cu and marine invasions

In order to examine the links that exist between Cu and the transfer of NIS, it is necessary to first evaluate the invasion process as a whole. For an organism to become a successful NIS (or invader) it must survive and persist through a series of discrete events that transport it away from its natural range to a new recipient location (Miller and Ruiz 2009). A successful invasion can be conceptualised as a result of four stages: (1) the entrainment of an organism by a human vector; (2) the transport of that organism outside of its natural range; (3) establishment of viable population(s) of the organism in the new environment; and (4) population spread away from the initial point of incursion (Carlton 1985; Richardson et al. 2000; Floerl and Inglis 2005). These processes can exert very strong selective pressures on individuals involved, with large numbers of potential NIS present in source locations generally reduced to only a small few able to survive the stressors involved and become invasive. Williamson and Fitter (1996) attempted to characterise the probability of successful species introductions *via* this series of events, and proposed the 'tens rule', whereby: (1) 1 in 10 of all the species transported to a new region (*via* natural or human-mediated dispersal) will survive in the wild; (2) 1 in 10 of the species surviving will establish and form self-reproducing populations; and (3) 1 in 10 of the species establishing will proliferate and spread to become pest species. When considering that hull fouling is one of the most common vectors for the transport of marine NIS, on-going research indicates that Cu has the potential to play a role in the transfer of NIS at every stage of the invasion pathway (Figure 1).

#### Entrainment and transport

The first two stages of the invasion pathway involve the entrainment and transport of a species to a new region by means of a human-mediated vector. Along with ballast water discharge, hull-fouling (ie biofouling) is now one of the most important dispersal mechanisms for marine NIS (eg Hewitt 2002; Gollasch 2002; Godwin 2003; Hewitt et al. 2004), and one where NIS and Cu interact very closely. In regions such as Australia, North America and Hawaii, it is estimated

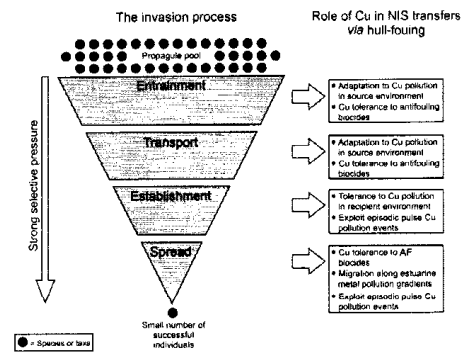


Figure 1. Diagram depicting the four stages involved in the invasion process and the influence that Cu may play in facilitating a successful species transfer *via* the common marine transport vector of hull-fouling.

that between 55 and 85% of recorded marine NIS are introduced *via* fouling on vessel hulls or other floating structures (Wasson et al. 2001; Eldredge and Carlton 2002; Hewitt 2002; Hewitt et al. 2004). This is particularly true for sessile invertebrate taxa such as bryozoans, ascidians, hydroids, serpulid polychaetes and barnacles (Hewitt et al. 2004). Species transfers *via* hull-fouling continue to occur despite the presence of AF biocides, such as Cu-based paints (Floerl et al. 2004; Piola and Johnston 2008b). While a proportion of these transferred species occur on hull areas that escape adequate AF coverage (eg dry-dock support strips, Coutts and Taylor 2004) or are prone to mechanical damage and/or high water turbulence (Otani et al. 2007), the indications are that at least some of these hull-fouling species have a tolerance to Cu biocides.

As early as the 1950s, scientists began noting marked Cu tolerance in some hull fouling organisms. When describing the proliferation of the previously unrecorded bryozoan species *Watersipora cucullata* (now known to be *Watersipora subtorquata*) in Australian waters, Allen (1953) noted '... it is exceedingly Cu-tolerant and can exist attached to fast ships for months ...'. He went on to state that based on the patterns of appearance and distribution of *W. cucullata* around Australia, '... the evidence suggests ship transport'. More recently, Floerl et al. (2004) found larvae of *W. subtorquata* and the arborescent non-indigenous bryozoan *Bugula neritina* were able to settle directly to surfaces coated with AF paints (including Cu-based coatings). Recruitment and growth on these surfaces was observed as little as 8 weeks post-submergence, with a percentage cover of

*W. subtorquata* similar to or greater than cover observed on non-toxic control panels. A similar study by Dafforn et al. (2008) compared the recruitment for native and NIS species to blank settlement panels with or without the nearby influence of Cu and TBT AF paints in boating harbours frequented by commercial and/or recreational vessels. After 10 months' submergence, the recruitment of native taxa was typically reduced by Cu, however, the total recruitment of NIS to Cu-influenced panels was 14–19% higher across all sites compared to control panels. Several NIS in particular showed considerable tolerance to Cu, with the early recruitment of the cosmopolitan encrusting bryozoan *W. subtorquata* and the calcareous tubeworm *Hydroides elegans* significantly enhanced by the presence of Cu. Given the primary dispersal mechanism for both these species has been closely linked to biofouling (Floerl et al. 2004; Pettengill et al. 2007), tolerance of Cu is a profound advantage. The recruitment of NIS to Cu-treated surfaces was generally greater in recreational boating harbours with a history of use by vessels treated with Cu-based antifoulants. The recreational boating harbours also had higher levels of Cu contamination in the water column than commercial sites (Dafforn et al. Forthcoming 2009). These studies support previous laboratory and field findings that detail substantial Cu tolerance in larvae and/or adults of NIS including *W. subtorquata* (Piola and Johnston 2006b, Forthcoming 2009) and *H. elegans* (Johnston and Keough 2003; Xie et al. 2005; Piola and Johnston 2008a). Studies examining marine algae have yielded similar findings to those seen for fouling invertebrate species. Ship-fouling populations of the marine algae *Ectocarpus siliculosus* and *Enteromorpha* (= *Ulva*) *compressa* have been found to exhibit greater Cu tolerance compared with populations sourced from an uncontaminated rocky shore location (Russell and Morris 1970, 1972; Reed and Moffat 1983). Similarly, a study of two species of *Ulva* from Korea found the alien *Ulva armoricana* to exhibit less effect of Cu toxicity than the native *Ulva pertusa* (Han et al. 2008).

In another study, it was demonstrated that small-scale (cm<sup>2</sup>) areas of unprotected settlement surface may be colonised by fouling taxa, even if these areas are surrounded by otherwise well maintained, newly applied Cu AF coatings (Piola and Johnston 2008b). Despite the likely exposure of unprotected ('scraped') areas to Cu from the adjacent painted surfaces, organisms were still found to recruit to scrapes as narrow as 0.5 cm wide. When scrape sizes were increased to widths of 1–2 cm, a much wider range of taxa recruited, including several well-known NIS, including the bryozoans *Aetea anguina*, *Bowerbankia gracilis* and *Bugula stolonifera*, the serpulid *H. elegans* and the colonial ascidian *Diplosoma listerianum*. This

study has obvious implications when considering hull maintenance regimes and vessel care. On a recreational or commercial vessel hull, areas such as hull sides, keels, and propeller and rudder wells may be highly susceptible to this type of minor damage during everyday operations (Lewis et al. 2003; Coutts and Taylor 2004). Such damage can be easily overlooked, and may result in the translocation of Cu-tolerant NIS by well-maintained vessels with an otherwise effective AF treatment.

The transfer of specific Cu-tolerant species is not the only scenario for Cu/NIS interaction. Research has found that some Cu-tolerant species, such as *W. subtorquata*, that are able to recruit and grow directly on Cu-treated surfaces, in turn have the potential to facilitate the transfer of less tolerant NIS. Wisely (1958) observed '... colonisation of an antifouling paint surface by *Watersipora*, which in turn is being utilised as a settling surface by the less resistant tubeworm *Hydroides norvegica*'. Similarly, Floerl et al. (2004) found that *W. subtorquata* acted as a 'foundation species' for fouling assemblages on vessel hulls, recruiting to undesirable (ie Cu-treated) surfaces and in turn acting as refugia for other less tolerant NIS, such as algae, serpulids and bryozoans (Figure 2).

#### Establishment

Having survived the entrainment and transport stages of the invasion process, Cu tolerance can further aid in the establishment of NIS within recipient environments. Harbours and estuaries are the primary source and recipient locations of NIS in marine systems (Ruiz et al. 1997), and also rank amongst the most contaminated environments worldwide (Hall Jr et al. 1998), with metal pollution often a major contributor (Kennish 2002). Cu in particular, is one of the most commonly occurring metal pollutants, originating

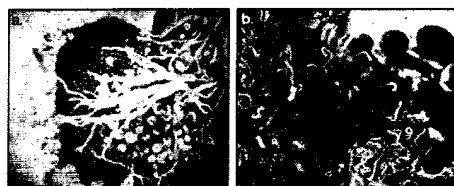


Figure 2. Images demonstrating how a Cu-tolerant foundation species can facilitate the establishment of less Cu-tolerant taxa. The cosmopolitan non-indigenous bryozoan *W. subtorquata* is shown growing directly onto a surface coated with Cu-based AF paint, in turn allowing the establishment of other taxa, including (a) the serpulid worm *S. australis* and spirorbid polychaetes, and (b) the encrusting bryozoan *S. errata* and serpulid worms. Photos: K. Dafforn.



from a wide range of sources including AF coatings (Warnken et al. 2004), industrial waste (Hall Jr et al. 1998), urban runoff (Pitt 2002), sewage discharge (Scanes 1996) and wood preservatives (Weis and Weis 2002). Since the banning of TBT there has been an increase in Cu levels around marinas and moorings associated with vessels using Cu AF paints (Claisse and Alzieu 1993). *In situ* studies have found that average passive flux rates of dissolved Cu for AF coatings on vessels may range between  $\sim 3$  and  $8 \mu\text{g cm}^{-2} \text{ day}^{-1}$  (Valkirs et al. 2003; Schiff et al. 2004; Finnie 2006), with factors such as coating age, hull cleaning activities and the presence of microbial biofilms all acting to decrease or enhance the release rates of AF biocides (Schiff et al. 2004; Yebra et al. 2006).

Cu has been shown to have detrimental effects on the health and success of many species and concentrations that are protective of 90% of marine species within estuarine communities (ie affect the long-term viability of 10% of species) have been estimated at  $\sim 6 \mu\text{g l}^{-1}$  (Hall Jr et al. 1998). Cu concentrations directly next to a Cu antifouled surface can reach up to  $\sim 100 \mu\text{g l}^{-1}$ , whilst dissolved Cu concentrations in the range  $60\text{--}85 \mu\text{g l}^{-1}$  have been recorded in polluted estuarine and open sea locations (Hall Jr et al. 1998; Stauber et al. 2000). The overall bioavailability and toxicity of Cu in marine aquatic environments is dependent on the presence and amounts of organic matter (eg detritus) and inorganic compounds such as iron and manganese oxides, which complex or adsorb metals (Stauber et al. 2000). Cu contamination can lead to fundamental changes in the structural composition of fouling communities (Weis and Weis 1996; Johnston et al. 2002). It is not difficult to imagine therefore, that the very same Cu-tolerance traits that allow some NIS to be transported to a new environment, for example by *via* hull-fouling, also serve to aid in their establishment within Cu-polluted habitats.

The marine alga *E. (=Ulva) compressa* exhibits differential Cu tolerance with populations from a Cu-enriched environment able to tolerate higher concentrations than populations from waters with reduced Cu contamination (Correa et al. 1996). When coupled with the higher Cu tolerance of ship fouling populations (Reed and Moffat 1983), this alga becomes a likely candidate for entrainment, transport and subsequent establishment *via* a Cu pathway.

A manipulative field study by Piola and Johnston (2008a) examined the effect of increasing Cu pollution loads on the diversity and distribution of fouling assemblages within two Australian harbours, concluding that NIS were competitively advantaged over native species in the presence of Cu. The spatial

dominance of NIS increased significantly with increasing Cu pollution loads at three of the four sites examined. This increase was coupled with a corresponding decrease in the diversity of native taxa, often resulting in fundamental changes to community structure. The only site where this trend was not observed was the site that had the greatest levels of background pollution (including substantial Cu input) and the highest background levels of NIS cover.

Laboratory-based studies provided further evidence for differential Cu tolerance in some common non-indigenous hull-fouling species compared to similar native taxa. Piola and Johnston (Forthcoming 2009) examined the effects of exposure to a range of Cu concentrations on the health and growth of two cosmopolitan non-indigenous bryozoan species (*W. subtorquata* and *Schizoporella errata*) and two native bryozoan species (*Celleporaria nodulosa* and *Fenestrulina mutabilis*). They found that NIS were able to maintain biomass, feed and grow better under Cu-conditions relative to the co-occurring native species, which deteriorated rapidly. Further, the NIS displayed much faster post-Cu recovery compared to the natives, suggesting that this trend for greater Cu tolerance may hold true under both chronic and episodic pollution regimes.

#### Range expansion and spread

The role of Cu in the spread of NIS within new environments may in part be a repeat, or addition, of the steps that brought the individual there in the first place. For example, resistance to Cu AF paints and hull-fouling can just as easily facilitate intra-regional spread of an organism as it can inter-regional translocation between bioregions (eg Forrest et al. 2009). A study by Wasson et al. (2001) examining the macrofauna of Elkhorn Slough (California) revealed 56 known exotic species in the estuary, a large number considering the relatively natural setting of the estuary, the lack of international shipping, and its distance from other major shipping hubs (ie San Francisco Bay, 150 km to the north). They concluded that 70% of the exotics found were associated with hull fouling as a mode of introduction, and intra-regional spread *via* recreational yachts was an important factor in their arrival and spread. Similarly, following preliminary investigations into the abundance and distribution of NIS in the World Heritage Property of Shark Bay, Australia, Wyatt et al. (2005) suggested hull fouling of recreational craft to be the most important vector for NIS transfer in the region.

The impact of Cu pollution on native communities may be sufficient to reduce the resistance of these communities to biotic invasion regardless of the

relative tolerance of NIS and native species (Clark and Johnston 2005). Biotic communities affected by pollutants can experience significant changes including the loss of rare/sensitive species, decreased species abundance, and changes to the size and age structures of populations (Schwinghamer 1988; Howells et al. 1990; Kennish 1997; Moran and Grant 1989). Such impacts invariably release valuable resources (eg space) that can be exploited by opportunistic species within the community (Johnston and Keough 2003). For example, Turner et al. (1997) used experimental epifaunal assemblages to investigate the effects of potential gradients of environmental stress arising from marina operations and boating activity. High levels of Cu and Zn were initially recorded in suspended sediments within marina sites, with levels decreasing along a gradient away from the marinas. Settlement panel arrays comprising established fouling communities were deployed along each pollution gradient and after 3–6 months, significant changes in epifaunal composition along the gradients were observed, with the most conspicuous change in assemblage structure being the loss of solitary ascidians at sites within marinas compared to sites furthest away. The non-indigenous bryozoan *W. subtorquata* was one of the most abundant species to occupy this newly created free space.

Cu tolerant NIS in harbours and estuaries may also take advantage of one-off episodic or 'pulse' pollution events to spread within a new environment. Cu pulses can enter coastal waters through a number of ways, including urban run-off, industrial, mining and metabolic wastes, AF paints and the corrosion of pipes (Mance 1987; Abel 1989; Paulson et al. 1989; Depledge et al. 1994; Pitt 1995; Fabris et al. 1999). As previously described in this review, numerous NIS across a range of taxonomic groups display superior Cu tolerance compared to similarly related native species (Piola and Johnston 2006b, 2008a, Forthcoming 2009; Dafforn et al. 2008). Further native taxa are often detrimentally affected by exposure to relatively low levels of Cu, often with prolonged recovery times post-exposure (Piola and Johnston 2006b, Forthcoming 2009). As such, short-term reductions in water quality from pulse Cu-pollution events may allow an opportunistic Cu-tolerant NIS to exploit temporary pollution-mediated impacts on native populations. This may provide a 'foothold' for expansion provided dependent factors such propagule availability and timing are also favourable (Clark and Johnston 2005). It should be noted, however, that the maintenance of Cu tolerance may be metabolically expensive and may not be essential to the persistence and spread of NIS that have established a foothold population (Piola and Johnston 2006a).

#### **Management of AF and related practices with respect to NIS**

Effective vessel AF practices remain the key to preventing NIS transfers. The age of an AF coating is often considered the most important factor governing the establishment of fouling assemblages on recreational vessels (Floerl and Inglis 2005; Floerl et al. 2005). TBT-based AF coatings had the potential to remain effective for up to 5 years between applications (Evans et al. 2000), and modern Cu-based SPC paints commonly used on deep-sea ocean-going vessels can achieve similar life-spans (Almeida et al. 2007). In contrast, however, conventional Cu-based coatings (ie soluble and insoluble matrix formulations) commonly used on many smaller coastal-going vessels (eg fishing vessels, tugs, and recreational craft) generally need to be applied every 9–18 months to ensure they are performing optimally (Lewis 2002; Almeida et al. 2007). In addition to regular maintenance, selecting the right paint for the type of vessel in question is crucial. Different types of Cu AF paints (such as self-polishing, soft ablative and hard non-ablative) all have advantages and disadvantages, with their suitability to provide adequate protection from fouling determined by such factors as the type of vessel, frequency of vessel use, season, geographic location, and the typical operating speed/profile of the vessel, as these affect polishing rate/ablation rate. The integrity of AF coatings on vessel hulls is highlighted as an important management priority for controlling species transfers (Piola and Johnston 2008b). Slight disruptions or minor damage in otherwise very effective AF coatings are sufficient to facilitate the recruitment and growth of fouling taxa. Such disruptions in AF coatings could easily occur through poor application of AF paints (eg uneven coverage, careless preparation, failure to follow manufacturer instructions), or as a result of everyday use (eg damage incurred from minor collisions, anchors, and groundings). Given that these areas of unprotected hull may occur on parts of the vessels that are not easily inspected (eg hulls, propeller wells, and keels) periodic haul-outs and dry docking may be necessary to ensure vessels that are new to a region are free of NIS, even if their AF maintenance histories records appear up to date.

One of the advancements in Cu AF technology since the banning of TBT coatings has been the use of organic booster biocides to supplement Cu oxide. However, these have their own suite of issues including the development of tolerant organisms within the target fouling community. Booster biocides such as Irgarol 1051, diuron, Sea-Nine<sup>TM</sup> and Cu and Zn pyrithiones, were generally introduced to target algal

slimes by inhibiting photosynthesis (Voulvoulis et al. 1999), but can also have a direct biocidal effect on invertebrates, as well as an indirect effect on invertebrate colonisation through biofilm modification and reduction (Keough and Raimondi 1996; Steinberg et al. 2002). Recent studies suggest that the use and accumulation of these biocides may also encourage the development of tolerance in marine communities and therefore reduce their effectiveness at preventing fouling and the transport of NIS. Petersen et al. (2004) found that high concentrations of Zn pyrithione resulted in increased growth of tolerant microbial species, and exposure to diuron (Molander and Blanck 1992) or Irgarol 1051 (Blanck et al. 2009) can induce tolerance in marine diatoms.

Alternatives to Cu-containing AF coatings are another option for preventing the transfer of Cu-tolerant NIS. For example, fouling-release coatings based on silicone (polydimethylsiloxane; PDMS) elastomers do not contain biocides. Such coatings are designed to 'release' accumulated organisms hydrodynamically, as the vessel moves through the water (eg Brady 2001; Candries et al. 2001; Kavanagh et al. 2005). However, such biocide-free fouling-release coatings will have a high potential for the introduction of alien species if used incorrectly. Watermann et al. (1999) found that removal of up to 90% of organisms on silicone coatings was directly dependent on the speed that the vessel was travelling. For some fouling-release coatings, voyage speeds of >20 knots may be required to remove growth (Brady 2001; Candries et al. 2005), making them better suited for use on relatively fast commercial vessels making regular voyages as opposed to intermittently used recreational vessels. While rigorous AF maintenance practices may not ensure that zero hull-fouling will accumulate on vessel hulls, when coupled with regular hull inspections they have the potential to reduce the number and frequency of Cu-tolerant species transferred *via* hull-fouling.

Traditionally, very little mention is made of water quality parameters as a means of controlling NIS. Current research however, highlights the importance of water quality as a management tool in the prevention or control of species introductions (eg Dafforn et al. Forthcoming 2009). In the past, it has been suggested that improvements to water quality in harbours and ports bearing high TBT burdens may promote the spread of exotic species, because reduced toxicity would allow a wider range of organisms to find port regions more suitable for colonisation (Minchin and Gollasch 2003). While this may have been true of TBT pollution, the comparatively less toxic nature of Cu presents a different scenario. Research suggests that rather than preventing the establishment of new

species, increased levels of Cu pollution in port and harbours would instead alter established selection regimes within these areas, inhibiting the competitive success of sensitive native taxa while facilitating the establishment and spread of more tolerant-introduced species. For example, Piola and Johnston (2006a) found evidence for adaptive expression and loss of Cu tolerance among different populations of the same species of NIS. This raises the possibility that in environments where background Cu levels are already high, vessel hulls may be more readily/heavily colonised by tolerant hull-fouling species. Possible ways in which water quality may be improved to reduce this risk include the adoption of alternative non-toxic antifoulants (eg Stupak et al. 2003), better management of the paint application and hull-cleaning processes, and the remediation of contaminated sediments to avoid the risk of toxicant release during resuspension events (Beck 1996; Turner et al. 1997).

Changes to shipping and port practices (both commercial and recreational) may also provide benefits for managing species introductions. Ideally, the mooring and docking of vessels need to be conducted in areas of low metal pollution. Otherwise, non-indigenous fouling taxa present on the hulls of moored vessels may be competitively advantaged over native species in the area. This could not only be achieved by reducing metal pollution loads within ports and marinas (eg sediment remediation, improved port and marina design, and strict pollution regulations), but may also be achieved by mooring medium and long-stay commercial vessels in port areas that receive good flushing. Similar approaches may be employed for managing species introductions stemming from recreational vessels. Entrainment of water in low flow, semi-enclosed marinas and boat harbours has been demonstrated to exacerbate the prevalence of non-indigenous hull-fouling taxa by limiting the dispersal of propagules, effectively increasing propagule pressure in these areas (Floerl and Inglis 2003). In a similar way, increased water residence times in marinas and embayments can also lead to the build up of AF biocides such as Cu (Warnken et al. 2004; Dafforn et al. Forthcoming 2009). Mooring recreational vessels in areas of high water movement and flow would result in more efficient dispersal and removal of pollutants, with fewer pollution-mediated impacts on native assemblages. Some degree of caution may need to be adopted if considering such a strategy; however, because this approach may also have the effect of dispersing non-indigenous larvae over greater distances, encompassing a wider range of native habitats instead of retaining them within disturbed areas.

Determining the Cu tolerance of marine species that are susceptible to entrainment and transfer *via*

vectors such as shipping (eg fouling assemblages in ports and harbours) may be a useful predictor of fouling species that have the potential to become invasive. Floorl et al. (2009a) measured the phylogenetic relatedness, ie similarity based on their evolutionary development or history, among marine bryozoans occurring in New Zealand ports and harbours, to determine whether the invasion process leads to higher or lower phylogenetic relatedness among NIS than that among native assemblages. They found phylogenetic relatedness among non-indigenous bryozoans was no different from that among natives in port environments, but instead native bryozoans occurring within ports had significantly reduced taxonomic distinctness relative to native New Zealand species occurring outside port environments, ie open coast. The authors suggest one possible explanation is that the distinct habitat characteristics associated with ports and harbours (eg high levels of pollution, dominance of artificial structures, and altered hydrodynamic regimes) have reduced the suite of native bryozoans present in New Zealand ports to a restricted subset of the entire native population, more tolerant of these conditions and hence more closely related to co-occurring NIS. Other studies within port environments support these findings, clearly demonstrating that some native taxa such as serpulids and barnacles exhibit some traits (eg Cu tolerance) that are commonly only associated with NIS (Dafforn et al. 2008, Forthcoming 2009; Piola and Johnston 2008a). If phylogenetic studies were to be combined with laboratory and field trials examining the Cu tolerance of native species, it may provide a useful toolkit for predicting future invaders or 'next-pests' amenable to transfer *via* hull fouling.

#### Future research directions

Recent progress in understanding the role of Cu pollution in species introductions and invasion biology has raised further questions and directions for research. One of the logical next-steps is the investigation of the evolution of metal tolerance in NIS. Detailed, multi-generational breeding experiments are required to determine the role of genotype and/or phenotypic plasticity in the development of metal resistance. Is metal tolerance in NIS induced during the organism's life, or is it a constant feature of certain genetic strains? Can this tolerance be passed on to offspring, thereby creating resistant propagule pools better adapted to colonise pollution disturbed environments? Can a vector such as hull-fouling, which has the potential to be highly selective for metal-tolerant species, be creating a growing body of 'super-invaders'? Knowledge of how rapidly metal tolerance can be acquired

and lost amongst populations of NIS may help to predict some of the mechanisms behind successful invasions.

Preliminary research has demonstrated differential Cu tolerance among different populations of the same NIS, with costs associated with Cu tolerance manifested as reduced competitive success under non-polluted conditions (Piola and Johnston 2006a). Marshall (2008) found similar evidence of costs associated with Cu tolerance. He showed that maternal colonies of the non-indigenous bryozoan *B. neritina* exposed to a Cu toxicant produced larvae that were larger, more dispersive and more Cu tolerant than toxicant naive mothers. However, the 'trade-off' for this Cu tolerance among offspring manifested as a decrease in post-metamorphic survival in non-polluted conditions, especially in the presence of high intraspecific competition. These costs and benefits associated with pollution adaptation make it a highly context-dependent characteristic, and one that might usefully be 'switched off' when no longer needed. If metal-tolerance is a costly and inducible response, then organisms may evolve a high degree of phenotypic plasticity that increases their fitness under changing environmental conditions (de Jong 2005).

Knowledge about the characteristics of the invader and the abiotic conditions in both the source and recipient region are essential to predict where future invasions are likely to occur (Miller et al. 2007; Hayes and Barry 2008). Correlative studies that compare the distribution of NIS within harbours and estuaries with local patterns of pollution may provide valuable insights into relationships between these two factors. For example, within the Sydney region of Australia, extensive sampling has generated a large amount of data on the metal loadings in sediments within two of the major harbours, Sydney Harbour and Botany Bay (Birch 1996; Birch and Taylor 1999). Both these estuaries have also been the focus of large-scale NIS surveys (AMBS 2002; Pollard and Pethebridge 2002). By employing geographical information system technology, it may be possible to correlate both sets of data to determine if patterns exist, for example whether NIS and Cu-pollution 'hot-spots' correspond, or whether different sources of pollution correlate with specific types of invaders. Preliminary work by Dafforn et al. (Forthcoming 2009) has identified several non-indigenous and native Australian species that were more abundant in areas of high Cu contamination. These findings highlight the need for countries to identify which of their native species exhibit Cu tolerance that might enhance their potential for transport and establishment. This would allow targeted management efforts to minimise the export of such species to other countries.

### Conclusion

The 'cost' of species loss through the use of efficient biocidal coatings (eg TBT SPC AF paints), vs the increased risk of species transmission by the use of less 'efficient' technologies (eg Cu; fouling-release coatings) to control fouling, needs to be assessed. In the case of TBT, this decision has already been made, with the detrimental environmental impacts resulting from its use deemed to be of greater importance than the question of whether cessation of its use may result in even greater ecological damage through the loss of native community composition and function in regions susceptible to invasion. In the absence of a readily available, effective alternative, Cu seems set to remain the most widespread commercial and recreational AF biocidal agent in use for the near future. Biocides such as Cu exert very strong selective pressures on both the target and non-target organisms, favouring individuals that have increased tolerance. When combined with a common transport vector for marine NIS, such as hull-fouling, the potential for such biocides to create competitively superior NIS (under polluted conditions) quickly becomes apparent. There is a growing body of evidence indicating that a repeating cycle of metal-polluted source environments, followed by metal-biocide influenced transport vectors, followed by equally polluted recipient locations, is leading to the evolution of a pool of highly metal-tolerant non-indigenous hull-fouling organisms that are successfully out competing less resilient native taxa worldwide. This transfer of metal-tolerant NIS is likely to continue into the near future, until: (1) alternative AF strategies, practices and regulations become more effective and widespread; (2) water quality issues (particularly concerning metal pollution) are addressed in ports, harbours and estuaries worldwide; and (3) a better understanding is gained of the nature and evolution of metal tolerance in marine fouling taxa, particularly with respect to differential tolerance among non-indigenous and native species.

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