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# Testing the efficacy of heated seawater for managing biofouling in ship's sea chests

## **Abstract**

Biofouling within sea chests may be more important than ballast water and hull fouling for dispersing certain non-indigenous marine species (NIMS). Despite this current Australian guidelines remain costly, ineffective or may increase the biosecurity risk of sea chests. This thesis tested the efficacy of a new heated seawater biofouling treatment technique on managing the biosecurity risks posed by sea chests. Chapter 2 provides a baseline assessment of time and temperature regimes required to achieve 100% mortality of secondary biofouling assemblages on Perspex settling plates placed in Port Kembla Harbour. Perspex plates were used because of relative ease to which fouling organism settle such artificial surfaces. Seawater heated to 40°C for 15 minutes was the minimum temperature and time required to achieve 100% biofouling mortality ( $F = 508.805$ ,  $p < 0.0001$ ). Interestingly 30°C had no significant impact on organism mortality ( $F = 2.6$ ,  $p = 0.115$ ). In total 1619 organisms were quantified, Bryozoans were the most prevalent group making up over 57% (935) of organisms/colonies identified. Other taxa included polychaeta (648), cirripedia (12), bivalvia (4), ascidians (8) and porifera (12). These findings show that moderately elevated seawater temperatures ( $> 40^{\circ}\text{C}$ ) are capable of treating 3 months of temperate marine biofouling. Future work might test temperatures between 30°C and 40°C determine minimum temperature and time regimes to achieve 100% biofouling mortality.

Chapter 3 tests the efficacy of Hull Surface Treatment (HST), a new biofouling treatment technology, for treating secondary and tertiary biofouling within sea chests. A mock sea chest (1 x 1 x 0.75m) was constructed for the HST trials. As was shown in the trials from Chapter 2 treatments of 40°C for 15 minutes were enough to ensure 100% mortality of secondary biofouling within sea chests. 40°C treatments however, did not show any significant difference from control treatments for tertiary biofouling ( $f = 3.000$ ,  $p = 0.114$ ). Both 60 and 70°C treatments were observed to cause 100% tertiary biofouling mortality ( $f = 13.102$ ,  $p < 0.0001$ ). These results show that HST is a viable option for treating the biosecurity risks associated with biofouling within sea chests. Currently HST cannot treat other vessel niche areas (without diver intervention), as such HST should be used in association other antifouling and defouling measures and maritime regulatory practices. Future Studies should focus on larger sea chests and on tropical assemblages.

## **Degree Type**

Thesis

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School of Biological Sciences

## **Keywords**

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# Testing the efficacy of heated seawater for managing biofouling in ship's sea chests

**Andrew Leach**

A thesis submitted in part fulfilment of the requirements of the Honours degree of Bachelor  
of Marine Science in the School of Biological Sciences,  
University of Wollongong, 2011

Supervisor: Dr Pia Winberg

Co-Supervisor: Dr Ashley Coutts

Industry Partner: Mr Chris Geater

## **Declaration**

The Information in this thesis is entirely the result of investigations conducted by the author, unless otherwise acknowledged, and has not been submitted in part, or otherwise, for any other degree or qualification.

Signed:

Date:

## Abstract

Biofouling within sea chests may be more important than ballast water and hull fouling for dispersing certain non-indigenous marine species (NIMS). Despite this current Australian guidelines remain costly, ineffective or may increase the biosecurity risk of sea chests. This thesis tested the efficacy of a new heated seawater biofouling treatment technique on managing the biosecurity risks posed by sea chests. Chapter 2 provides a baseline assessment of time and temperature regimes required to achieve 100% mortality of secondary biofouling assemblages on Perspex settling plates placed in Port Kembla Harbour. Perspex plates were used because of relative ease to which fouling organism settle such artificial surfaces. Seawater heated to 40°C for 15 minutes was the minimum temperature and time required to achieve 100% biofouling mortality ( $F = 508.805$ ,  $p < 0.0001$ ). Interestingly 30°C had no significant impact on organism mortality ( $F = 2.6$ ,  $p = 0.115$ ). In total 1619 organisms were quantified, Bryozoans were the most prevalent group making up over 57% (935) of organisms/colonies identified. Other taxa included polychaeta (648), cirripedia (12), bivalvia (4), ascidians (8) and porifera (12). These findings show that moderately elevated seawater temperatures ( $> 40^{\circ}\text{C}$ ) are capable of treating 3 months of temperate marine biofouling. Future work might test temperatures between 30°C and 40°C determine minimum temperature and time regimes to achieve 100% biofouling mortality.

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## Chapter 1: Introduction

It is now recognised that over 429 marine species have been introduced into Australain water ways (Hewitt and Campbell, 2008). Many of these species are problematic fouling species that affect both commercial industries (e.g. aquaculture and shipping) and natural environments. Biosecurity (biological security) is the protection of native environments and commercial ventures from the potentially harmful impacts of introduced marine species. Human mediated incursions of non-indigenous species (NIS) into new environments has been recognised as a major mechanism causing environmental change around the world (Vitousek et al., 1996). The introduction of non-indigenous marine species (NIMS) can have catastrophic environmental, economic and social consequences (Carlton, 1996, Pimentel et al., 2000, Hewitt, 2003).

### Impacts of Non – Indigenous Marine Species

The environmental impacts of NIMS are not completely understood, but include species, population, community or entire ecosystem effects (Parker et al., 1999). It is estimated that the introduction of NIS is considered to be the second most important cause of native species loss globally (Vitousek et al., 1996). NIMS can include low impact and cryptic introduced species, as well as marine “pests” that have a larger effect on the marine ecosystem. Obvious impacts on native communities include predation, competitive exclusion and habitat modification (Pimm, 1989). For example the New Zealand screwshell, *Maoricolpus roseus*, is affecting soft sediments in south-eastern Australia. *Maoricolpus roseus* covers soft sediments with it’s hard shell, which can provide structures for marine fauna (including other exotic species) to settle onto (Hewitt et al., 2005). The increase in *M. roseus* has been linked to a decrease in a threatened, native scallop species, *Gazameda gunnii*, which occupy the same beds (Patil et al., 2004). *Maoricolpus roseus* may reduce the numbers of scallops via direct competition for food and space as they are a filter feeding species with similar habitat requirements (Bax et al., 2003a). Other introduced species impacts include changes to predator-prey relations, changes in food web-structures, hybridisation, parasitism and (in the case of NIMS) bioturbation (Pimm, 1989). The New Zealand screwshell’s resultant shells provide effective homes for hermit crabs where sandy

sediments were previously inhibited by surface macrobenthos (scallops dominating the substrate, providing no viable homes for hermit crabs). The resultant increase in predation is expected to have a large impact on the post settlement survival of native screwshells and scallops (Bax et al., 2003a).

The economic and social impacts of NIMS can include threats to human health in the case of pathogenic microorganisms or toxic species in the food chain. For example harmful algal blooms have increased in frequency, many species of which are invasive around the world (Van Dolah, 2000), these blooms can have significant direct health impacts with humans as well as the local environment. Other costs include impacts on the productivity of industries dependent on the health of the marine environment including fisheries, aquaculture, tourism, marine infrastructure and shipping. In the Black Sea an invasive ctenophore, *Mnemiopsis leidyi*, is considered to have caused the collapse of the coastal fishing industry worth millions of dollars annually (Shiganova, 1998). NIS have been estimated to cause losses up to \$120 billion annually in the US alone (Pimentel et al., 2000). Such economic effects have immediate social impacts through decreased employment and flow on economic downturns in human communities. Other social impacts may be through declines in community welfare due to the decreased quality of the native environment.

Characteristics of successfully established species vary depending on the vector and the environment being colonised however some common attributes have been observed (Table 1). Both the European Green Crab (*Carcinus maenas*) and the North Pacific Seastar (*Asterias amurensis*) have successfully established themselves in southern Australia. Both of these species have a large native range (indicating high tolerance to physical variation) in which they have a high abundance, they are both mobile with a broad diet, they each have a highly fecund and dispersive life history strategy and are able to function in a wide range of environments (Thresher et al., 2000, Byrne et al., 1997). Often the environment being colonised displays signs of disturbance, alterations to ecological, biological, chemical or physical states change the susceptibility of recipient regions to invasion (Carlton, 1996, Dafforn et al., 2009).

Table 1: Characteristics of Successful Invaders

Successful Invaders	Unsuccessful Invaders
Large native range	Small native range
Abundant in original range	Rare in original range
Mobile	Sedentary
Broad Diet	Relatively restricted diet
Short generation times	Long generation times
Able to shift between r and K strategy	Unable to shift
Much genetic variability	Little genetic variability
Gregarious	Solitary
Female able to colonize alone	Female unable to colonize alone
Larger than most relatives	Smaller than most relatives
Associated with <i>H.sapiens</i>	Not associated with <i>H.sapiens</i>
Able to function in a wide range of physical conditions	Only able to function in a narrow range of physical conditions

(adapted from Ehrlich, 1989)

The total number of NIMS in Australia has increased from 55 reported in 1990 (Pollard, 1990) to over 250 species reported in just under a decade. Over 170 of the 250 species reported have been found in Port Phillip Bay, Victoria alone (Hewitt et al., 1999). In 2008 the total number of NIMS recorded in Australian waters was 429 (Hewitt and Campbell, 2008). This increase reflects a global and domestic increase in the frequency of vessel movements, as well as changes in the shipping trade (Perrings et al., 2005), changes in environmental conditions that facilitate introductions (Carlton, 1996, Glasby et al., 2007) and greater awareness of biosecurity as an issue. A combination of government initiatives and a cultural shift towards environmental awareness has lead to a greater awareness of NIMS in general, but it wasn't until the establishment of the Centre for Research on Introduced Marine Pests (CRIMPS) within the CSIRO in the late 1990s that lead to a greater understanding of the state of NIMS in Australian waters. The introduction of genetic technology has seen the development of new tools being used to better inform population structure as well as sources of species (Turon et al., 2003).

When a marine pest is established it is usually difficult to eradicate (Thresher and Kuris, 2004). Therefore, a preventative approach is the preferred means of treating marine pest

incursions, made possible by management of the vectors (mechanisms of dispersal) that marine pests use (Lafferty and Armand, 1996, Perrings et al., 2005). In 2009, the Australian Government started The National Introduced Marine Pests Coordination Group (NIMPCG), with the purpose of leading the implementation of the National System for the Prevention and Management of Marine Pest Incursions (NSPMMPI) to address the management of NIMS. The NSPMMPI is a group of measures aimed at: 1) preventing or minimising the arrival of marine pests; 2), providing an emergency response; and 3) managing and controlling established marine pests (NSPMMPI, 2009). Supporting the major components of the NSPMMPI are four aspects; an ongoing national monitoring program to provide early detection of new pests, industry and community targeted communication and education, targeted research and development of policy and new management measures, and finally continual evaluation of the effectiveness of the National System. Whilst this is a great step forward in the prevention of invasive alien species the guidelines for commercial vessels are voluntary and are lacking in rigour or have been shown not to be completely effective (Coutts and Dodgshun, 2007).

## **Vectors for Non-indigenous Marine Species**

NIMS are transported internationally in a variety of ways (Table 2) including shipping, recreational boats and fishing, aquaculture and the aquarium trade (Bax et al., 2003b, Carlton, 1987, Jousson et al., 1998). Marine shipping has long been known to be a vector of NIMS (Allen, 1953), and is considered to have been the greatest contributor to unintended marine invasions (Carlton, 1987). Biofouling is the major mechanism by which NIMS are introduced, around 46.2% of all marine incursions are a result of marine vessel biofouling (Hewitt and Campbell, 2008). As well as environmental costs of biofouling there are also major economic costs. Increased fuel consumption has been noted to be the primary economic cost attributed to biofouling as increased friction on the ship means the engine has to be run harder and for longer to maintain the same speed. It is estimated that for Arleigh Burke-class destroyer DDG-51 the US navy will spend over \$56 million per annum as a result of increased fuel costs due to biofouling (Schultz et al., 2011). The increased cost estimated by the Office of Naval Research for the entire US naval fleet is \$500 million USD. It

is clear that there are both environmental and economic reasons for the management of vessel biofouling.

**Table 2: Vectors for NIMS relocation**

<b>Source</b>	<b>Vector</b>
Commercial Shipping	Ballast Water Hull Fouling Niche Areas
Aquaculture and Fisheries	Intentional Release for Stock Management Gear, Stock or Food movement Discarded nets, floats, traps. Trawls, etc Discarded live packing materials Release of Transgenic Species
Drilling Platforms	Ballast Water Hull Fouling
Canals	Movement of species through locks due to water motion or active swimming
Aquarium Industry	Accidental or intentional release
Recreational Boating	Hull Fouling
Dive Practices	Snorkelling and scuba gear
Floating debris	Discarded Plastic debris

(adapted from Bax et al., 2003b)

The Australian Government has classified three stages of biofouling: primary, secondary and tertiary. Primary biofouling is the formation of a slime or biofilm consisting of bacteria and microscopic algae. Secondary biofouling usually includes hard encrusting animals such as barnacles, bryozoans, serpulid polychaets and may also include algal tufts and mobile amphipods (NIMPCG, 2009). Tertiary biofouling builds up on the secondary layer and generally consists of larger, more competitive organisms, such as sponges, large ascidians, bivalves and large algae. This system is used to determine the risks of biofouling communities containing NIMS.



Within commercial shipping there are a variety of mechanisms and biofouling points that are known to facilitate NIMS incursions (Table 2), including ballast water and hull biofouling (Carlton and Geller, 1993) as well as some cryptic niche areas. Historically, the majority of work being undertaken to prevent marine incursions has focused on the management of marine pests transmitted via ballast water and biofouling, and included management practises of shipping routes and harbour ports, ballast water exchange and hull cleaning regulations (ANZECC, 1997, AQIS, 2008) as well as the NIMPCG management practises described above. Less attention has been given to the perceived threat of many other vectors (Table 2) and ship niche areas (Bax et al., 2003b), nor biosecurity strategies to reduce those threats. Niche areas are parts of the ship considered to be protected or a refuge that can provide surface for the settlement and survival of marine organisms. They include internal water systems, sea chests, the rudder hinge, propeller, bilge keel, bow thrusters and dry-docking support strips (Coutts and Taylor, 2004, NIMPCG, 2009).

Niche areas on ships can provide significantly different conditions as a vector for NIMS compared to ballast water or hull surfaces. The enclosed small spaces, lack of effective antifouling paints and elevated temperatures provide many niche areas with suitable conditions for a variety of species and larger organisms that might not survive on a hull surface or in ballast water. For example the European Green Crab, *Carcinus maenas*, has been observed to be present within sea chests at the adult stage of its lifecycle (Coutts et al., 2003). The transport of adult organisms is particularly hazardous due to their ability to release gametes or viable larvae into the surrounding environment (Godwin, 2003). An organism whose propagules may not survive the transportation process in ballast water may be able to be transported to new areas in the adult stage via a ship's niche areas.

## **Sea Chests**

A predominant niche area are the sea chests – recesses built into a ship’s hull below the waterline for the purpose of increasing water pumping efficiency for ballast, engine cooling and fire fighting purposes (Figure 1). The size, shape and number of sea chests vary, as a

general rule the larger the ship the greater the size and number of sea chests required, with many large vessels having multiple upper and lower sea chests. Sea chests are covered with a steel grate to prevent large debris from entering the sea chest during ballast pumping when close to the substrate. This grate however, does not prevent the uptake of marine organisms. Seachests are a high biosecurity risk for the marine shipping industry, due to the inherent difficulties with treating an enclosed and often inaccessible space (Coutts et al., 2003). Sea chests are a niche area that provides a harbour for both planktonic and sedentary species (Coutts et al., 2003). In 2006 large populations of the invasive mussel, *Mytilis galloprovincialis* were found in the sea chests of the South African National Antarctic Programme supply vessel, the SA ‘Agulhas’ whilst the vessel was dry docked (Lee and Chown, 2007).

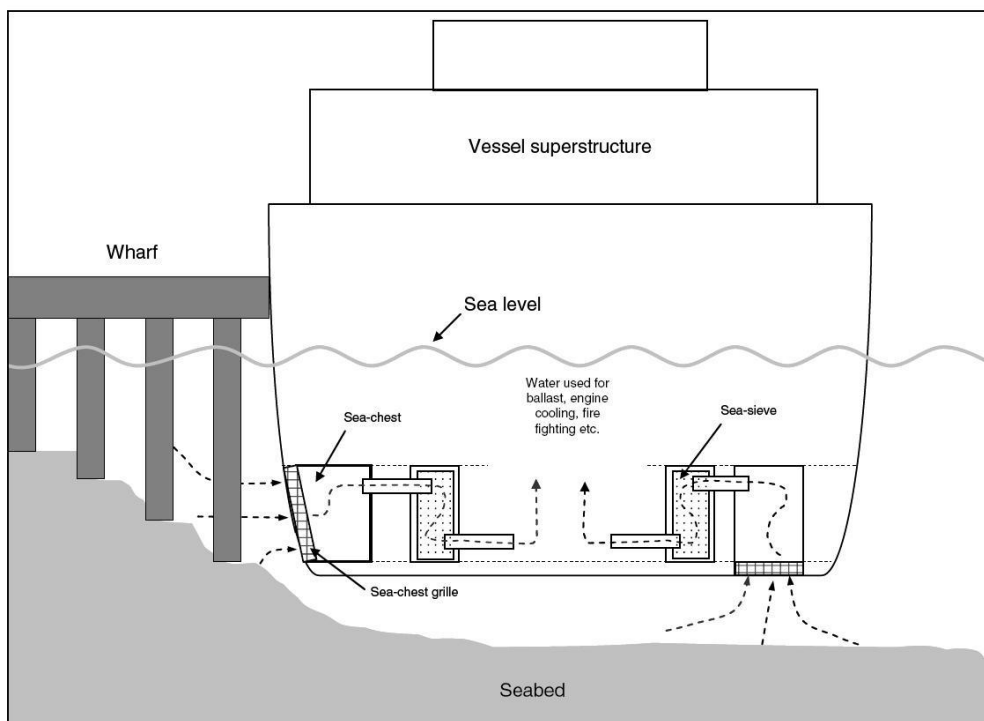


Figure 1: Schematic diagram of a vessel's sea-chest system (Coutts and Dodgshun, 2007).

In 2007 Coutts and Dodgshun identified some 150 different organisms within sea chests from a variety of vessel types, routes and geographic regions. The swimming crab *Carupa tenuipes* was found inside a ship's sea chest. Whilst this crab is usually known to inhabit coral reef and rubble in the western indo-pacific area it is not native to New Zealand where it was observed in a sea chest. *Carupa tenuipes* is now recognised to be established in the eastern Mediterranean where it inhabits rocky bottoms (Pancucci-Papadopoulou et al., 2009). The great number of species found in sea chests can be attributed to; 1) the fact that organisms are usually sucked into the sea chest from the water column, neighbouring wharfs or even the seabed; and 2) Larvae also seek out dark areas with increased water flow. Sedimentary organisms sucked up from the surrounds of the ship do not always settle as larvae, as such they may be more resilient to the affects of anti-fouling measures. As a result pseudo-communities can exist within the sea chest with multiple species, at varying stages of their life cycle with varying life histories being transported.

The presence of adult mobile organisms within sea chests is particularly concerning and may indicate that sea chests are of greater importance than ballast water or hull fouling for dispersing certain marine species (Coutts and Dodgshun, 2007). For some NIMS transport by conventional mechanisms (ballast water and hull-fouling) does not fully explain their presence in new communities, particularly mobile holo-planktonic organisms. Sea chests have been put forward as a possible vector for animals who could not have survived transport via a ship's hull or ballast water. The age of the *M. gallaprovincialis* inside the sea chest of the south african antarctic supply vessel showed that they had survived multiple travels to the antarctic (Lee and Chown, 2007). This tells us that *M. gallaprovincialis* is capable of short-term survival in polar conditions or that whilst contained within a ship's sea chest fouled organisms are protected from the conditions outside of the ship. Within sea chests organisms are provided with continuous renewal of food and oxygen, elevated temperatures (as a result of heat transfer from the engine) as well as the complete lack of hydrodynamic forces experienced on the ship's hull (Coutts and Dodgshun, 2007). These factors make the sea chest a particularly hospitable place for organisms to settle or seek refuge and then in turn are transported all over the world.

## **Biofouling Management in Sea chests**

Table 3 shows the key advantages and disadvantages of past and present biofouling management strategies for the hull of a ship. Current Australian guidelines for treating sea chests recommend the use of anti-fouling paints and the use of steam blow-out pipes where applicable (NIMPCG, 2009). In certain circumstances mechanical systems (scraping or rotating brushes) are also allowed for sea chests. Scraping or brushing has two major environmental issues; 1) the organism is not retained and if viable it can settle and successfully establish, and 2) it does not account for propagule release that may occur during organism disturbance. Anti-fouling paints do not perform as they do on external structures like the hull. This is due to the paint being subject to both minimum and maximum extremes of water-flow. Accordingly organisms can establish themselves on areas of premature paint degradation or in pockets of the sea chest where water movement is minimal and the paint is ineffective (Coutts and Dodgshun, 2007). Mechanical defouling systems may also increase the rate at which the paint degrades. Research also suggests that whilst antifouling paints are effective against sedentary organisms they are less effective against the mobile and adult organisms experienced in sea chests (Coutts and Dodgshun, 2007).

Table 3: Key Advantages and disadvantages of past and present biofouling management systems

Anti-fouling system	Key advantages	Key disadvantages
Dry Docking	Arguably the Safest- Removes vessel from water, ensuring fouling organisms and propagules are retained (Yebra et al., 2004).	Extremely difficult and expensive. Must be planned in advance and can mean months out of the water. May inadvertently introduce marine pests when ship is removed from water (Coutts et al., 2010).
In-water defouling (Scraping and Brushing)	Cheaper than dry-docking, saves on time. Fouling retention techniques can retain over 90% of fouling mass.	The biofouling organism is not retained and is then left to settle (Woods et al., 2005). Does not account for propagule release. Banned in Australia for hull use. Can damage antifouling paints.
Hot Water Treatments (e.g. HST)	Relatively cheap, can be undertaken when ship arrives in port, “environmentally friendly”, doesn’t release organisms. Doesn’t affect paints.	Time consuming – around two days (one day if two units used) to treat a whole ship. Treatment of particular niche area required diver intervention.
TBT self-polishing copolymer (SPC) coatings	Most effective broad spectrum AF biocide developed, long lifetime (5 years)	Impacts on non-target species, human health risks, half-life of days in seawater, but months – years in sediments depending on environmental conditions. Now banned in Australia
Tin-free SPC coatings	Effective against range of invertebrate foulers, long lifetime (5 years)	Cu and booster biocide impacts on non-target species, Cu persistent in marine environment (depends on pH, salinity and dissolved organic matter – also determines toxicity)
Tin-free conventional coatings	Effective against range of invertebrate foulers	Short lifetime (12– 18 months), Cu and booster biocide impacts on non-target species, Cu persistent in marine environment (depends on pH, salinity and dissolved organic matter – also determines toxicity)
Booster biocides	Effective against a range of bacterial, algal and fungal foulers	Impacts on non-target species, e.g., algae, seagrasses, corals, invertebrates, some persistent in marine environment
Foul-release coatings	strength of fouling attachment, do not leach, no or low toxicity, potential long life (10 years)	Only self-clean on high speed (>15 knots)/high activity vessels, or otherwise require regular cleaning, susceptible to abrasion damage
Biomimetics	Natural alternatives “environmentally friendly”	Not commercially available yet, difficult to source adequate supply of compound

Heated water treatments are seen as a practical way forward for the biofouling management of not only sea chests but of other niche areas on a ship where antifouling paint is impractical or unfeasible (Flemming, 2002, Coutts and Dodgshun, 2007). The idea of heated water treatments to act against biofouling is not completely new (Graham et al., 1977), however most of the research addresses tertiary biofouling on the cooling systems of coastal power stations (Rajagopal et al., 1995, Thiyagarajan et al., 2000). Heat treatments in the form of sprays are unlikely to be effective against thick shelled organisms such as mussels or oysters. Nel et al. (1996) found that when exposed to 70°C for 40 seconds did not raise the core temperature of the invasive Pacific Oyster, *Crassostrea gigas*, above 24°C. However experiments by Rajagopal (2005) observed *C. gigas* experienced 100% mortality when immersed in 42°C water after 60 minutes. Rajagopal (1995) also found that at a temperature of 39°C, the tropical green mussel *Perna viridis* showed 50% mortality after 59 minutes and 100% mortality after 73 minutes. The age and size of *P. viridis* strongly affected the mortality rate. Heated water has been used to treat the Asian Clam *Corbicula Fluminea* around power and chemical plants (Jenner and Janssen-Mommen, 1993). The invasive stalked ascidian *Styela clava* was killed after immersions in 60 and 70°C for 15 and 10 seconds respectively (Minchin and Duggan, 1988). Forrest and Blakemore (2006) whilst treating *U. pinnatifida* also described the hot water tolerance of the greenlipped mussel *Perna canaliculus*. Measuring mussel mortality as “mussel attachment” it was found that 30 mins at 55°C was sufficient to ensure 0% mussel attachment. Hot seawater hasn't been typically applied to ships because heat treatment is difficult to implement in maritime conditions unless the fouling environment can be isolated e.g. ballast water.

Heated seawater has been tested for its application to ballast water (Mountfort et al., 1999, Rigby, 1997), with the majority of research focused on heat trials lasting hours. The results of these surveys found that to successfully treat ballast water a minimum temperature of 35°C for 20 hours is required. Mountfort et al. (1999) recommended to treat larvae of *C. gigas*, 50°C for 2.7 minutes would be required. The short term treatment at higher temperatures (50-80°C) has been found to achieve 100% mortality for zooplankton that (Quilez-Badia et al., 2008). Hot water treatments have been shown to be particularly effective for the treatment and eradication of the internationally recognised pest The Asian Kelp, *Undaria pinnatifida* (Wotton et al., 2004, Forrest and Blakemore, 2006). The sea chest

is especially problematic as it can also harbour mobile juvenile and adult organisms; as such any heat treatment regime applied would have to ensure the mortality of tertiary fouling and mobile organisms such as adult shell fish and crabs. When some invertebrates experience a temperature increase they are inclined to release gametes (Minchin, 1987), as a result any heating technology will have to seal the sea chest during treatment to ensure no propagules are released. If hot water treatments are to be used to treat the biosecurity risks posed by sea chests then the development of technologies and protocols that can both: 1) maintain a high temperature within a sea chest for an extended period of time and 2) be able to ensure nothing is released during treatment application.

### **Hull Surface Treatment (HST)**

One of the current heated seawater technologies developed to treat hull surfaces is Hull Surface Treatment (HST). HST is a patented technology and a registered trademark owned by Commercial Diving Services (Australia) Pty Ltd. HST uses thermal shock to treat primary biofouling, such as copper resistant algae, on ship hulls that is predominately responsible for significantly increased hydrodynamic drag (Thomas & Coffey Marine, 2010). HST works by using a specially designed applicator (Figure 2) which forms a soft seal on the hull and applies heated seawater to the ships hull provided by a larger boiler placed on a small neighbouring vessel. The applicator is moved up and down the ship's vertical hull until the majority of the vertical hull has been treated (Figure 3). HST does not clean hulls by physical disturbance like traditional rotating brush systems do; but rather it kills the biofouling organisms *in situ*. Dead primary biofouling remains on the hull until the ship leaves port and is ground down in open water (Thomas & Coffey Marine, 2010).

As stated above the biosecurity risks posed by sea chests are notoriously hard to manage. The proposed technique for HST treatment is to place the applicator over the grill of the sea chest and fill the sea chest with heated sea water for a prolonged period of time (Figure 4). HST is a promising new hot water technology for the treatment of biofouling, however whilst hot water is adept at controlling the biosecurity risks associated with biofouling on vessel hulls no research has been conducted on heated seawater's ability to treat biofouling within sea chests.



Figure 3: HST applicator with inhalant and exhalent hoses attached.



Figure 2: HST Vessel and Applicator treating a ship.





Figure 4: Proposed HST applicator placement for the treatment of sea chests.

## Project Objectives

To address the limitations of effective treatment for secondary and tertiary biofouling of sea chests identified above, the objectives of this study included:

- 1) To establish and identify biofouling taxa from a commercial shipping port in a shaded environment that would be reflective of biofouling in commercial vessel sea chests.
- 2) To determine the most effective exposure time and temperature of heated water to cause 100% mortality to biofouling on settlement plates.
- 3) To test the application of selected temperature and time exposures to hot water in a mock sea chest to cause 100% mortality of both secondary and tertiary biofouling species.

The research in this thesis contributes to the research and development effort to strengthen the first strategy of the National System for the Prevention and Management of Marine Pest Incursions – prevention of marine pest incursions.

## **Chapter 2: Temperature and time exposure tolerance of primary and secondary biofouling taxa from shaded environments**

### **Methods**

#### **Settlement Plate Deployment**

For the first objective of establishing a representative community of biofouling organisms from shaded environments on experimental units, both site and settlement unit substrates were considered. 174 Perspex settlement plates (200 x 160 x 3mm) were deployed in Port Kembla Harbour under Jetty Number 4 (-34°28'32", 150°54'40") (Figure 6) on April, 4 2011.

In temperate regions of Australia's east coast, biofouling organisms that can exist in shaded environments include bryozoans, sponges, colonial and solitary ascidians, algae, barnacles and tube polychaetes (Glasby, 1997). These organisms readily settle on artificial substrates such as Perspex (Perrett et al., 2006). The Perspex plates had been aged in previous settlement studies and were acid washed in 10% hydrochloric acid to remove any influence of previously settled organisms. To ensure that only one side of the settlement plates were settled, paired plates were secured back to back and secured with cable ties at 4cm intervals along specially constructed PVC pipe racks (Figure 5). The plates were orientated vertically, this aided in minimising the presence of algae (personal statement, Johnston). In total, three rectangular racks were constructed (1800 x 2000mm) with a cross pipe for added rigidity. Holes were drilled in the pipes to allow the racks to flood and sink and also be easily pulled out of the water.

The racks of settlement plates were in a shaded environment under a commercial jetty (# 4) in Port Kembla Harbour, to mimic the shaded environment of sea chests. The racks were suspended with the vertical plates approximately 2 meters below the low water mark and approximately 6 meters above the sediment – a relevant depth to reflect the depth of sea chest grates on ship hulls. The settlement plates were then left to foul for approximately three months; however plates were checked fortnightly to ensure plates were retained and to make qualitative assessments of organism growth. The 12 control plates used for the

temperature and exposure treatments were used to quantify the relative abundance and composition of phyla that had settled on the plates. Organism identification was undertaken during the survivorship assessment (below).



Figure 5: Settlement plate racks before deployment.



Figure 6: Jetty Number 4, Port Kembla Harbour (-34°28'32", 150°54'40") (Google Earth 6.0, 2011 )

## Time and Temperature Exposure

Settlement plates were recovered after three months of immersion on July 1, 2011 and prepared for exposure to temperature exposure trials. Eight temperature and time regimes were chosen (Table 4) based on the review of temperature tolerance ranges of biofouling organisms from the literature as well as the hull application temperatures of the Hull Surface Treatment commercial applications.

**Table 4: Table 3: Treatments, Controls and Replications of Temperature Study (C = Control Temperature, 15 or 30 = treatment time, RSP = Control Temperature and Control Time and P# = plate number)**

<b>Treatment Number</b>	1	2	3	4	5	6	7	8	C15	C30	RSP
<b>Temperature (°C)</b>	30	30	40	40	60	60	70	70	Control	Control	Control
<b>Time (min)</b>	15	30	15	30	15	30	15	30	15	30	Control
<b>Replications</b>	4	4	4	4	4	4	4	4	4	4	4

In an effort to minimise disturbance to the biofouling organisms before treatment, all treatments were undertaken on site under Jetty number 4 in Port Kembla Harbour (Figure 6). Plates were removed from racks, photographed, tagged then placed in a shaded, holding tub filled with seawater of ambient temperature. Plates were submerged into one plastic tub used for each treatment of temperature and time exposure. To ensure a steady and controlled temperature supply of hot seawater water, the boiler on T&C Marine’s HST vessel was used to deliver heated seawater with a hose attached. When the desired water temperature was achieved in the treatment bath the plates were submerged for the allocated treatment time. Water temperature was monitored using the live thermocouples at the end of the HST hose, and was also verified using digital temperature loggers inside the treatment tub. Controls were exposed to unaltered seawater inside the treatment tubs for the allotted amount of time, and were used to establish any effects on survival from the handling process.

After treatment, settlement plates were re-attached to the racks and resuspended over night to simulate real world conditions where the animals would be exposed to ambient

seawater again after treatment. This also allowed organisms that may have survived the treatment a chance to recover before being transported. The “RSP” plates were collected from the settling racks, photographed, tagged then resuspended to control for the effects of disturbance as a result of transportation to the lab. The following day (2/7/11) the treated plates were collected from the settling racks and transported to the Shoalhaven Freshwater and Marine Centre where they were suspended in aquaria in preparation for survivorship assessment.

### **Survivorship Assessment**

The survivorship of biofouling organisms on the treated settlement plates was assessed using a rapid visual technique (Woods et al., 2005). Plates were chosen at random from the aquaria system, and 4 quadrates (50 x 40 mm, 6.25% of the plate surface area) were randomly chosen for analysis. A Leica M26 dissecting microscope at 2.0X optical zoom was used to identify and quantify each organism greater than 2mm in length to the level of phyla or class and assessed for viability using the same guidelines used by Woods et al. (2005) (Appendix 1). Due to the plates being placed in aquaria and the inherent difficulties with counting, mobile organisms were observed but not quantified.

### **Statistical Analyses**

Relationships between organism viability and temperature/time were tested using a one-way ANOVA. The effect of temperature on organism viability meant that no interaction was tested. Control data was explored for homogeneity and normality using SPSS Statistics 17, Release Version 17.0.1 (SPSS Inc., 2008, Chicago, IL, [www.spss.com](http://www.spss.com)). Species richness and diversity measures were carried out using PRIMER, Release Version 6 (PRIMER-E, 2006, Plymouth). Multivariate analyses of community structure between control plates, as well as analysis of community structure changes due to temperature were tested using PRIMER-E (Clarke, 1993).

## Results

### Ecological Findings

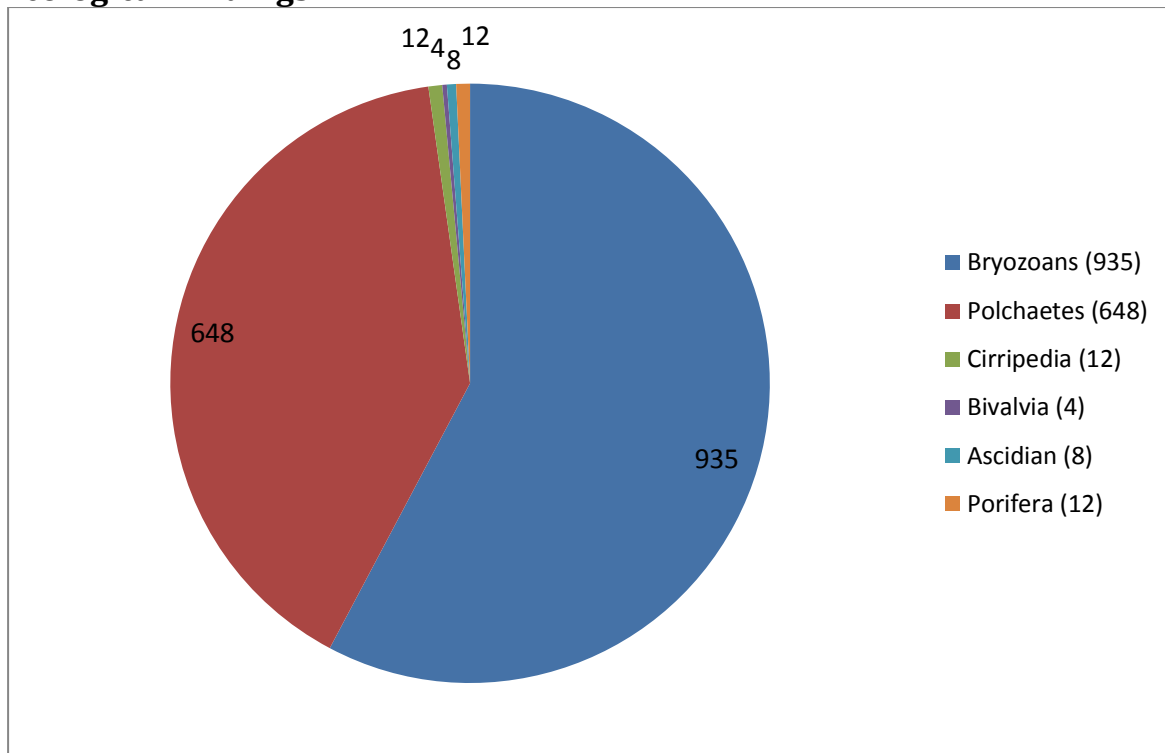
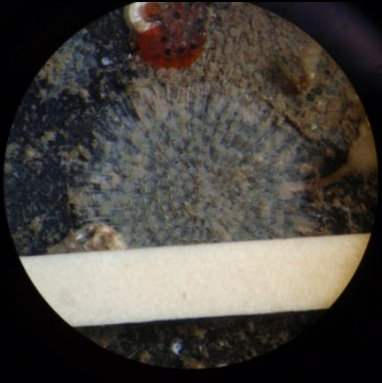







Figure 7: Total number of each taxa identified across all plates in the temperature and time exposure tolerance trials

1619 organisms or colonies in total were counted across all treatment plates (Figure 7). An average of 2.3 phyla (SE = +/- 0.38) were identified in each of the 36 quadrats across the 12 control plates (Figure 10). Bryozoans made up over a half (57%) of organisms with 935 individuals or colonies identified across all plates. Further taxa included polychaeta, 40% (648) and the remaining 3% of organisms comprised of cirripedia (12), bivalvia (4), ascidians (8) and porifera (12). Per quadrat on control plates bryozoans averaged 60% (S.E.  $\pm$  4.6) of organisms observed whilst polychaetes averaged 22% (S.E.  $\pm$  4.2) of organisms per quadrat (Figure 8). Table 5 shows examples of the 6 taxa identified during the survivorship assessment.



Table 5: Observed examples of each taxa identified in time and temperature trials.

Organism	Observed Example	Organism	Observed Example
<p><b>Bryozoa</b> (Red bryozoan species is <i>Watersipora arcuata</i>)</p>		<p><b>Bivalvia</b></p>	
<p><b>Polychaeta</b></p>		<p><b>Ascidia</b> (Ascidian Test, no alive ascidians photographed)</p>	
<p><b>Cirripedia</b> (no photo of Cirripedia taken, example photo provided- <i>Tesseropora rosea</i>)</p>		<p><b>Porifera</b></p>	

(Barnacle photo taken from Davey, 2000)

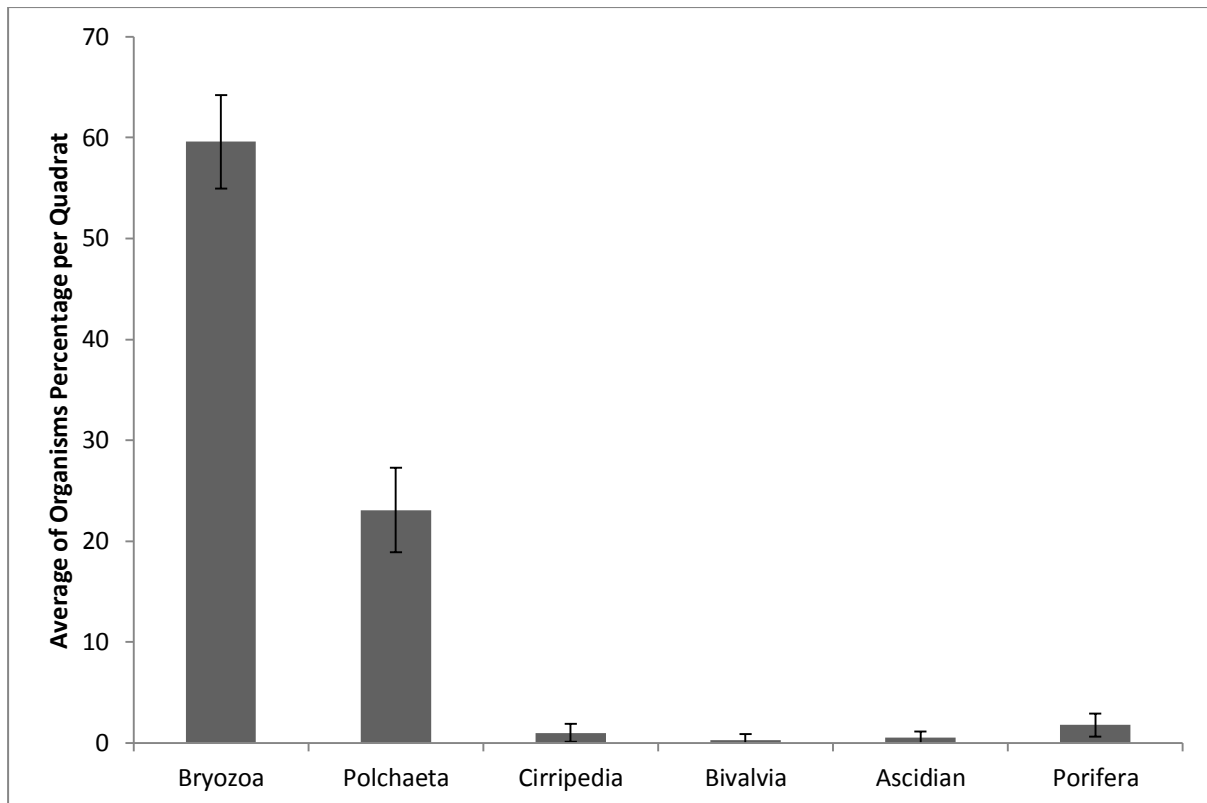


Figure 8: Average percentages of organisms of each phylum that settled on control plates after three months of fouling time between April and June 2011 ( $\pm$ S.E.).

Biofouling assemblages were consistent across the plates with no significant differences in their abundance (Figure 9) ( $f = 1.542$ ,  $p = 0.159$ ) taxonomic richness (Figure 10) ( $F = 1.479$ ,  $p = 0.184$ ) or diversity (Shannon  $H'$ log $e$ ) (Figure 11) of organisms settling on settlement plates ( $F = 1.472$ ,  $P = 0.185$ ). Multivariate assemblage analysis (Figure 12) also showed that there was no significant difference in assemblage abundance and relative composition between the control plates (ANOSIM Global  $R = 0.059$ , sig = 15%).



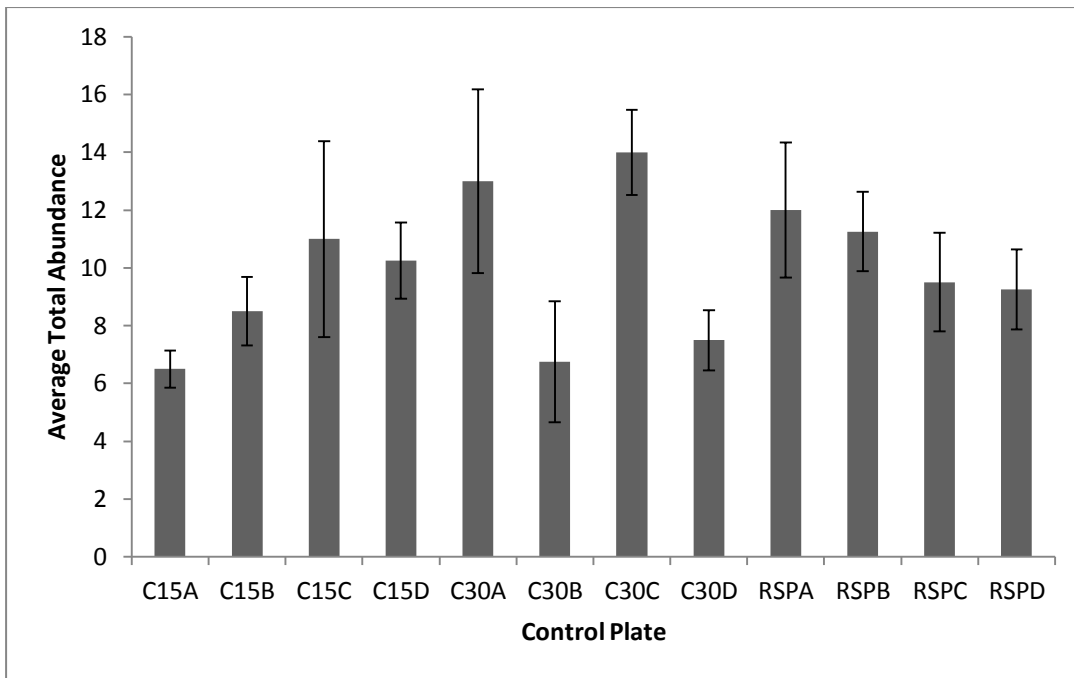


Figure 9: Average total organism abundance on control plates (±S.E.).

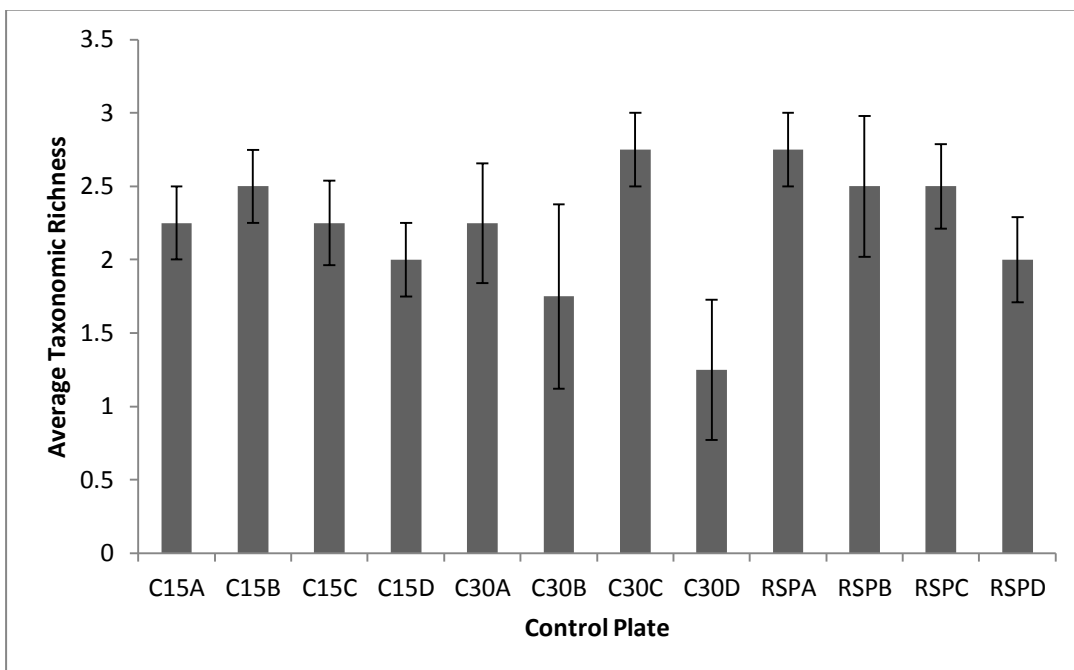


Figure 10: Average Taxonomic richness across control plates (±S.E.).

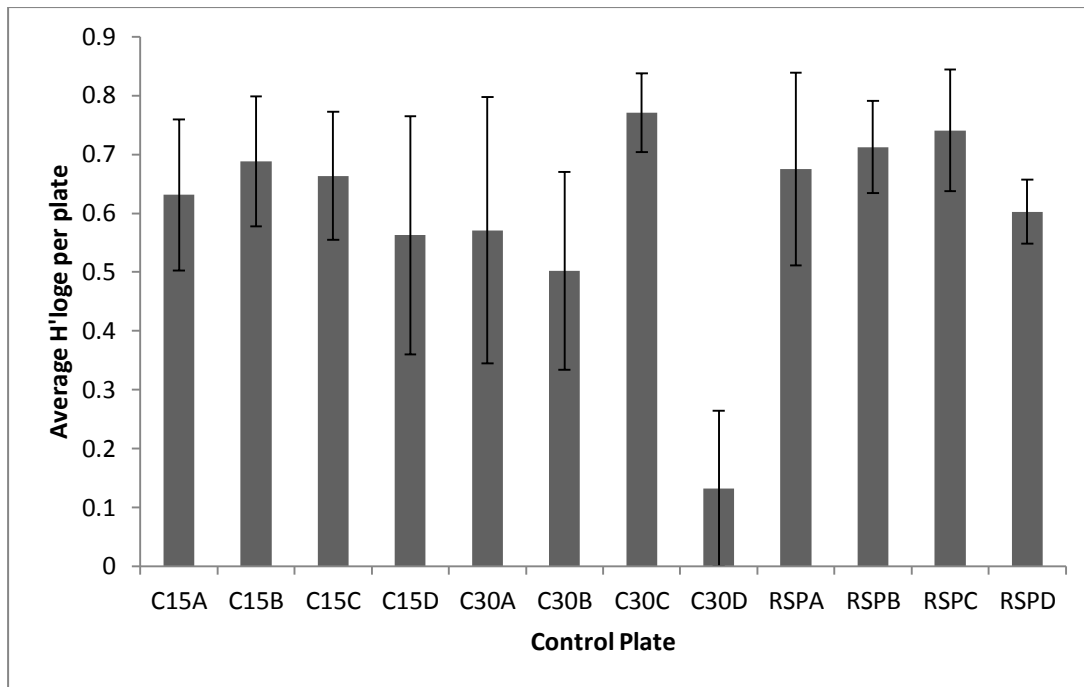


Figure 11: Average H' loge measure of diversity of control plates (±S.E.).

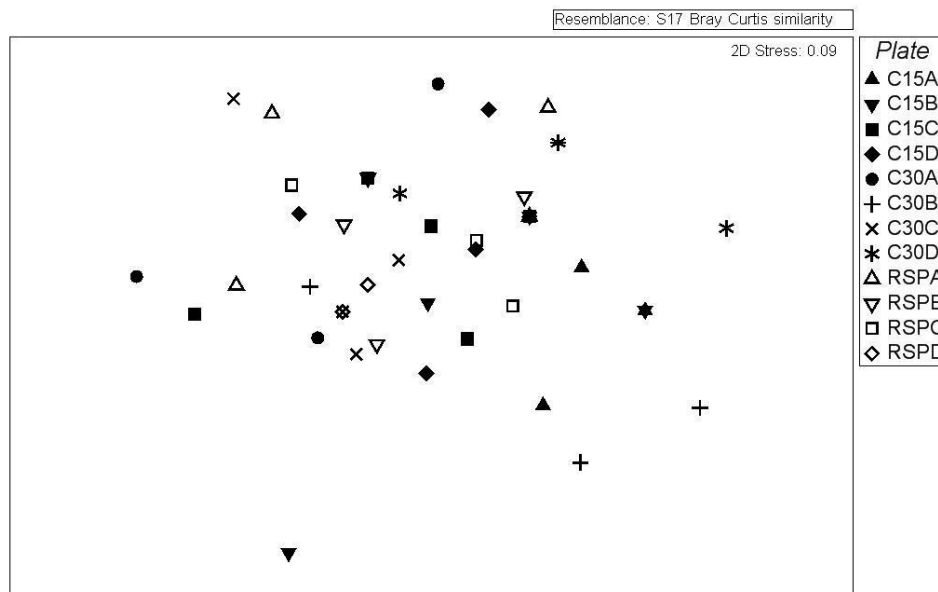


Figure 12: Multidimensional scaling ordination of plate averages assemblages on control settlement plates. No transformation applied to data and the comparison represents both composition and relative abundance of assemblages.

## Temperature Effects on Viability

Temperatures of 40°C and above had 100% effective mortality impact on organism viability ( $F = 508.805$ ,  $p < 0.0001$ ) (Figure 13). There was no significant impact of 30°C treatments on any of the assemblage taxa ( $F = 2.6$ ,  $p = 0.115$ ). At the higher temperatures, even the shortest time exposure of 15 minutes resulted in total mortality. Polychaetes were observed to have time to release propagules when disturbed during survivorship assessment at 30°C and control temperatures. Many polychaete tubes were empty, whilst some had the bodies of the worm hooked to the point at the opening of the tube, possible evidence of some animals trying to escape at the 40°C temperature range. Mobile amphipods were present during some plate assessments. There was no difference between assemblages found on control plates and plates treated with 30°C water (Figure 14). There was no significant difference in survivorship for control plates subject to treatment methods and those plates resuspended after photographing and tagging (Figure 13).

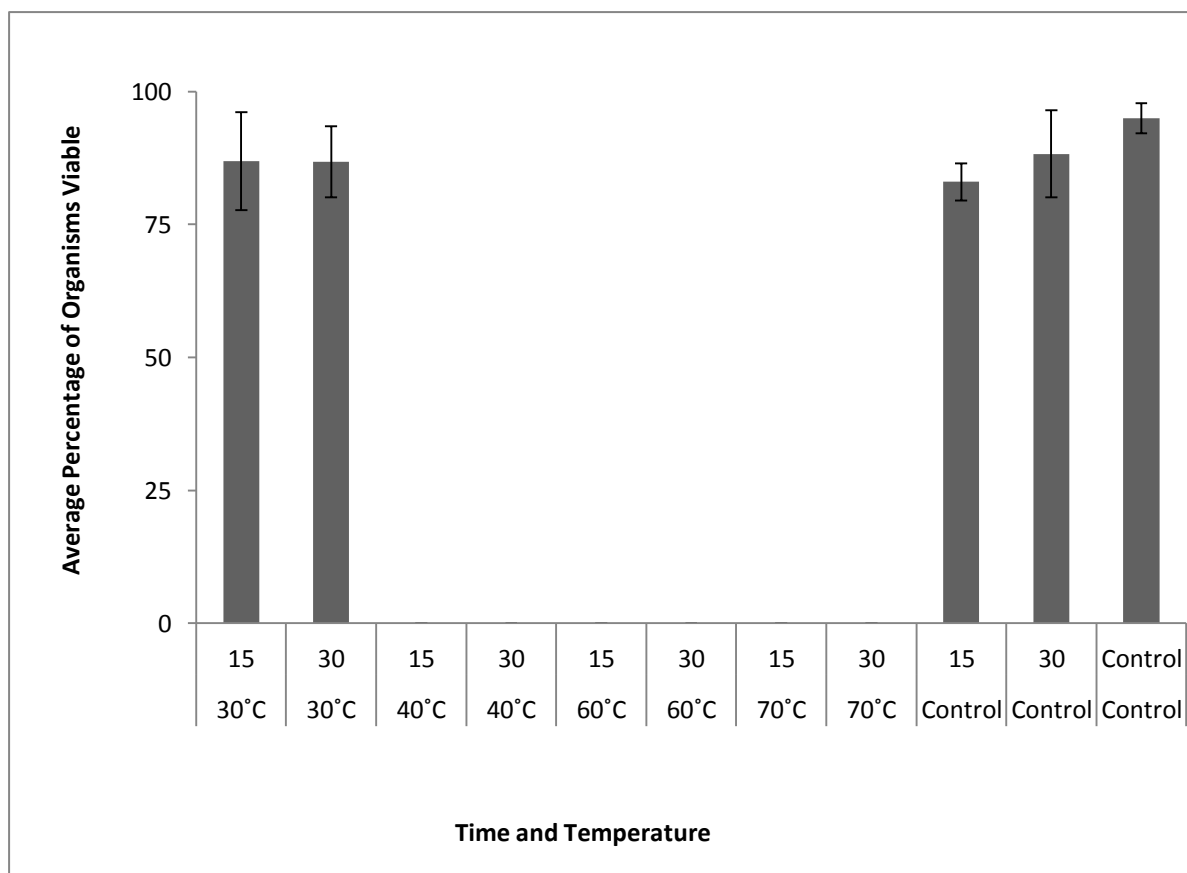
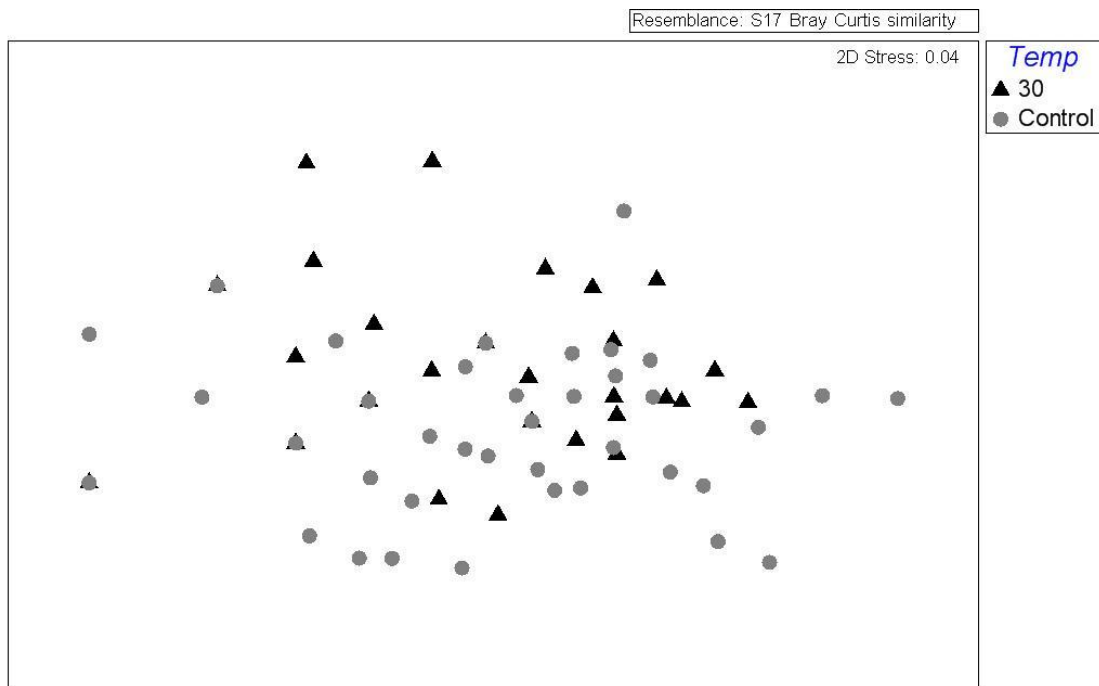


Figure 13: Average viability of organisms after treatment regimes (±S.E.).



**Figure 14: Multidimensional scaling ordination of assemblages in each quadrat on 30°C treatment and control settlement plates. No transformation applied to data and the comparison represents both composition and relative abundance of assemblages.**

## **Chapter 3: Sea chest trials**

### **Methods**

#### **Mock Sea Chest Construction**

To determine if HST has the ability to ensure that the required elevated water temperatures can be achieved and maintained throughout the sea chest the construction of a mock sea chest was necessary. The sea chest was 1 x 1 x 0.75m (W x H x D) and constructed from mild steel sheets welded into a cube (Figure 15). The grate or grill was approx. 0.45 x 0.45 m and positioned in the centre of the front-facing side. The front-facing side was also hinged to allow access to the inside of the sea chest and to allow the sea chest to be opened in the water (Figure 15). The front facing panel was also fitted to a latch to ensure a watertight seal (Figure 19). Both the steel sheets and welds were constructed from mild steel and were painted in order to reduce corrosion. The paint was applied by first using a galv etch (a non-corrosive liquid that leaves the surface of metals rough for paint to adhere without the need of a primer), then all surfaces were coated with an enamel-based paint. On the top side of the sea chest two exhalent valves were welded, this allowed for the sea chest to be completely flooded with seawater before the treatments began (Figure 19).

In reality sea chests are on the sides of maritime vessels, the inside of the sea chest is submerged in water whilst the five external sides are usually surrounded by air within the engine room of vessels. As such 20 mm marine ply was screwed to the outside of the sea chest for insulation in an attempt to simulate real world conditions (Figure 19). Twelve screws, 150 mm were screwed into the corners and sides of the front facing and back facing panels of the sea chest; these were used to fasten the settling plates and temperature loggers to the sea chest (Figure 14, 16). Hooks were placed in the in the sea chest to fasten mussel bags during the trials.



Figure 15: Sea Chest after paint with screws for settlement plates and temperature logger placement.

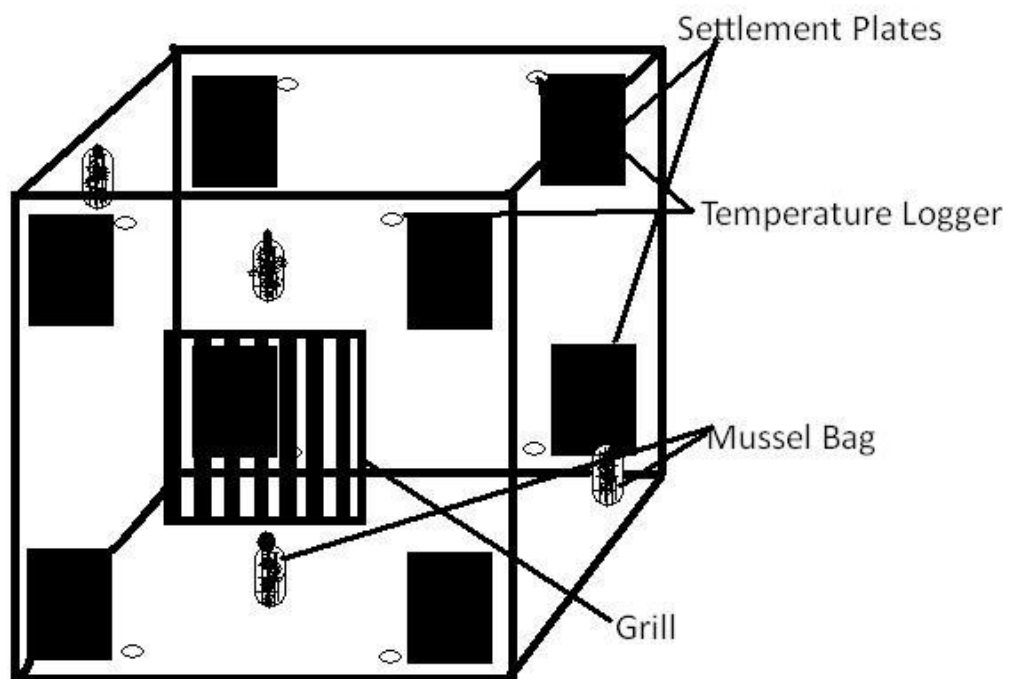


Figure 16: Placement and Locations for plates, temperature loggers and mussel bags within mock sea chest.

## Replica Applicator Construction

A model replica of the HST technology applicator was constructed. The applicator was constructed using marine ply with valves used to and sealed holes cut into allow for the attachment of inhalant and exhalent hoses as well as the insertion of thermo couples to allow for live temperature readings (Figure 17, Figure 19). The applicator was fastened to the front wall using 6 magnets (as with the real world applicator) (Figure 17, 19). The applicator was sealed around the grill to allow ensure no hot water escaped from the grill. An extendible hose was delivered into the sea chest without the grill impeding the hot water flow in anyway (Figure 18). Handles were also fastened to the replica applicator to ensure the hinged front facing side of the sea chest can be easily opened and accessed to attach treatment plates internally (figure 17).



Figure 17: Replica applicator with handles, hose valves and thermo couple inlets



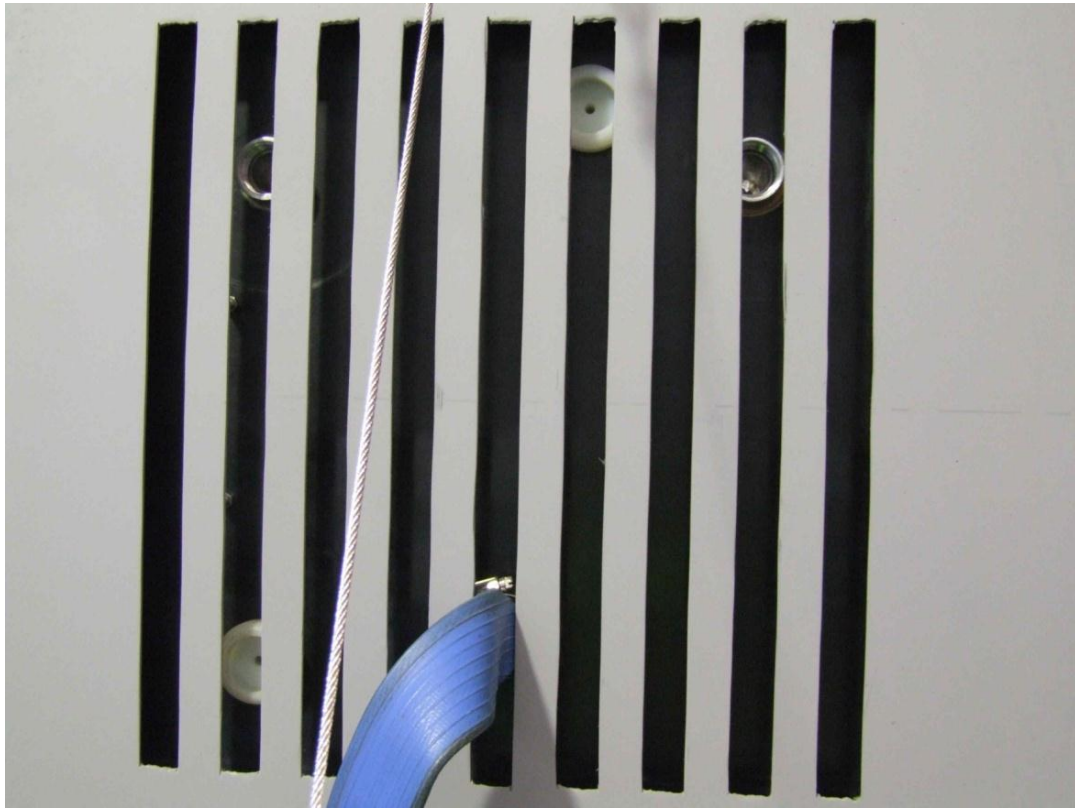


Figure 18: Inside of replica HST applicator through inside of mock sea chest grill

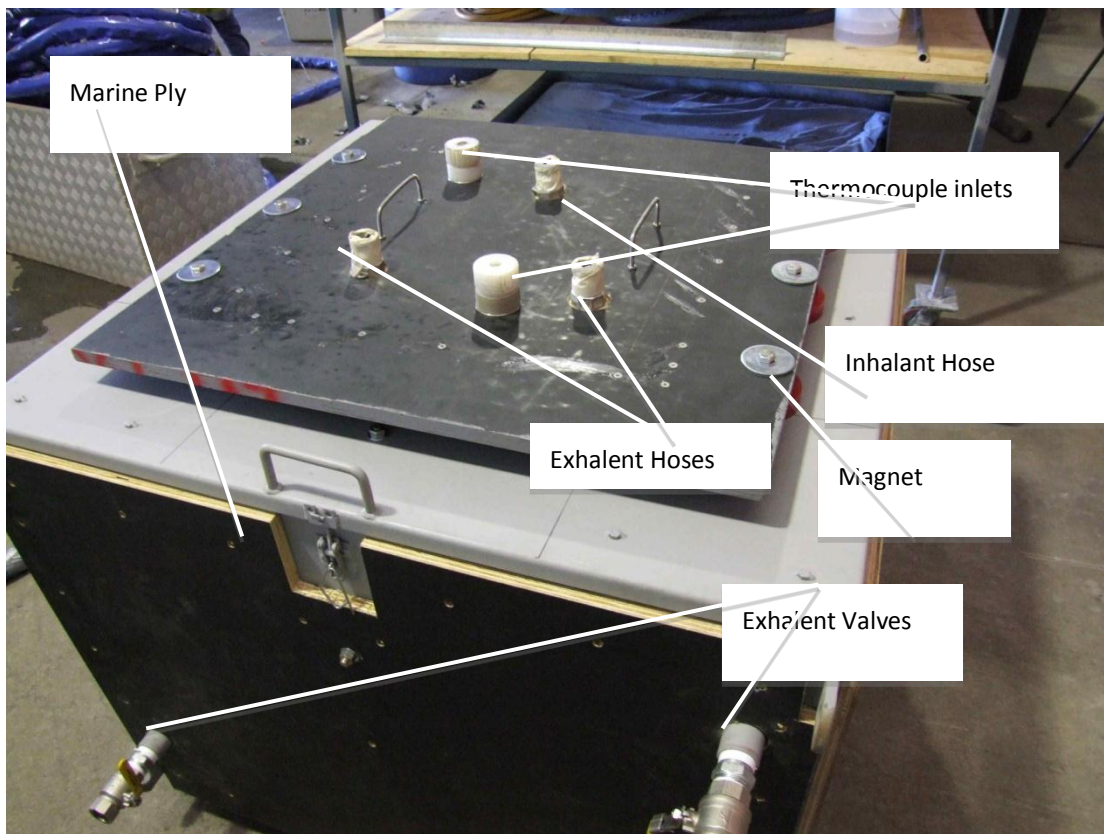


Figure 19: Finished mock sea chest with replica applicator attached



## Hot water treatment application to biofouling organisms

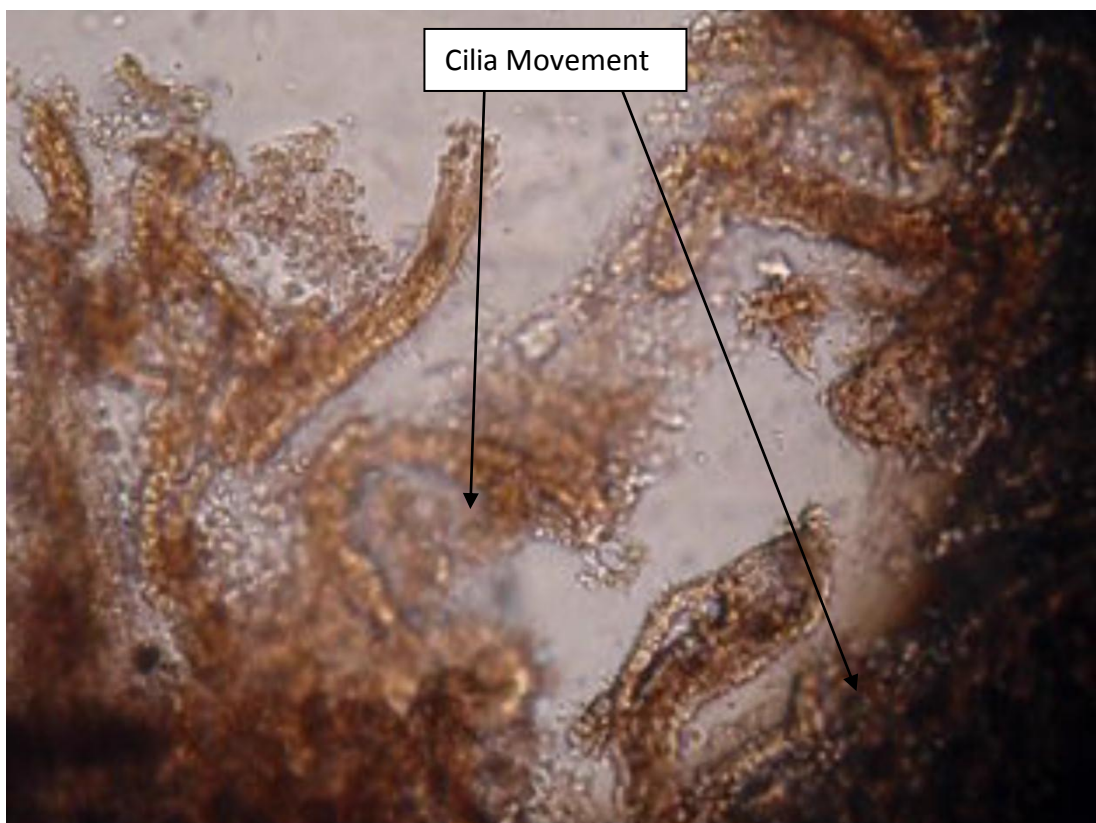
Sea chest trials were undertaken *in situ* under Jetty number 4 in Port Kembla harbour (-34°28'32", 150°54'40"). The trial took advantage of the settlement plates from the settlement racks in Chapter 2. Trials took place on August, 4 2011- four months of biofouling. The mock sea chest completely filled with 750 litres of water was too heavy for manual handling; as a result a sea crane was required for lifting and submerging the sea chest.



Figure 20: Sea crane provided by Thomas & Coffey

To test the HST technology's ability to treat biofouling that may be present in sea chests, mussels were used. On the support beams and ropes under Jetty Number 7 in Port Kembla, two mussel species were growing, *Mytilus edulis* (a NIMS itself) and *Trichomya hirusta*. A week before the main trial day, 15 each of *M. edulia* and *T. hirusta* were collected and placed in onion bags then resuspended on the settlement racks. This would allow the byssal threads of the mussels to attach to the onion bags and be used for the trials. Due to some

changes in the number of trials and number of mussel bags used in each trial more mussels were collected on the trial day. This meant that for many mussels used in the trail the byssal threads had not attached to the onion bag used in treatment. As the mussels are not ingesting a biocide it was assumed that the lack of byssal thread attachment would not have an affect on heated seawater trials. In total 30 *M. edulia* and 55 *T. hirusta* were collected. At least 4 mussels were placed into each bag, 4 bags were used in each trial, placed on screws throughout the sea chest as shown in Figure 14. The mussel viability was assessed using the guidelines used by (Woods et al., 2005). When mussel's viability was questionable, a biopsy of the mussel's gill was examined under a microscope. The mussel was determined to be alive if cilia movement was observed.



**Figure 21: Cilia movement as observed under an optical microscope during mussel survivorship assessment.**

Four temperature regimes were selected based on the pilot temperature and time of exposure trials and applications of the commercial HST technology. The temperature regimes were; 70°C and 60°C at 10 minutes each and 40°C for 15 and 30 minutes. Controls

for this trial were placed inside the sea chest and submerged for the maximum treatment time (30 minutes), the sea chest being flooded with untreated seawater.

An initial run was undertaken with temperature loggers placed in each corner of the sea chest to determine the temperature variability within the sea chest and the maximum temperature sustainable. In addition, thermocouples were also used to obtain a live reading of the temperatures achieved. The onboard boiler heated the seawater to its maximum temperature (approximately 85°C), the sea chest was then submerged, and all air was released. After the sea chest was submerged for 5 minutes the HST unit was initiated, when the maximum temperature was reached inside the sea chest it was maintained for 20 minutes. The sea chest was then lifted to the surface, opened in the water and brought aboard the HST boat and temperature logger data was analysed.

To apply the hot water treatment to plates inside the mock sea chest, the settling plates and mussels were brought up from settlement racks, photographed and tagged for treatment identification and then placed in a pre-treatment holding tub in ambient seawater. The sea chest was placed onboard the HST vessel and the temperature loggers, settlement plates and mussel bags were then secured inside the sea chest (Figure 16). The sea chest was then lowered into the water with the top exhalant valves open to release all air inside the sea chest. The valves were then closed and the sea chest was submerged for 5 minutes before the treatment started. This ensured all settlement plates, loggers and mussels' experienced the same water temperature before treatment. After the 5-minute rest period, the sea chest was heated until desired temperature was achieved on the live reading from the HST unit, this was maintained for the specific treatment time. When the treatment was complete, the sea chest was brought to the surface, opened by a diver and then brought aboard the HST vessel for the next treatment. At the end of each treatment, mussel bags and settlement plates were placed in a post-treatment tub in ambient seawater awaiting transport back to the laboratory at the end of all treatments. After the settlement plates and mussel bags were transported to the laboratory they were suspended in marine aquaria at 18°C with aeration. No nutrients were added to the water and the tanks were kept out of direct sunlight whilst the organisms were assessed for viability following the same

survivorship assessment procedure for the pilot temperature and time of exposure trials (Chapter 2).

### **Statistical Analysis**

Mussel species numbers were not equal nor were there always the same number of mussels in each bags, as such; no analysis of the relationship between mussel species and mortality was explored. Relationships between mussel viability and temperature and time were tested using a one-way ANOVA. The effect of temperature on mussel viability meant that no interaction was tested. Control data was explored for homogeneity and normality using SPSS Statistics 17, Release Version 17.0.1 (SPSS Inc., 2008, Chicago, IL, [www.spss.com](http://www.spss.com)). Species richness and diversity measures were carried out using PRIMER, Release Version 6 (PRIMER-E, 2006, Plymouth). Multivariate analyses of community structure between control plates, as well as analysis of community structure change from 3 to 4 months were undertaken using PRIMER-E (Clarke, 1993).

During the course of these trials concessions were made to account for the added cost of this study. This meant that one trail day was available to conduct the main mock sea chest trials. The decision was made to test a variety of temperatures and use multiple settlement plates within the sea chest as the replicates within the sea chest. This meant that only one plate experienced one treatment in one corner of the sea chest, essentially the trails were pseudo replicated. Ideally a minimum of three runs of each temperature regime would have been conducted to ensure a maximum confidence. These added trails would have exceeded the allowable budget for an honours project.

## Results

### Ecological Findings

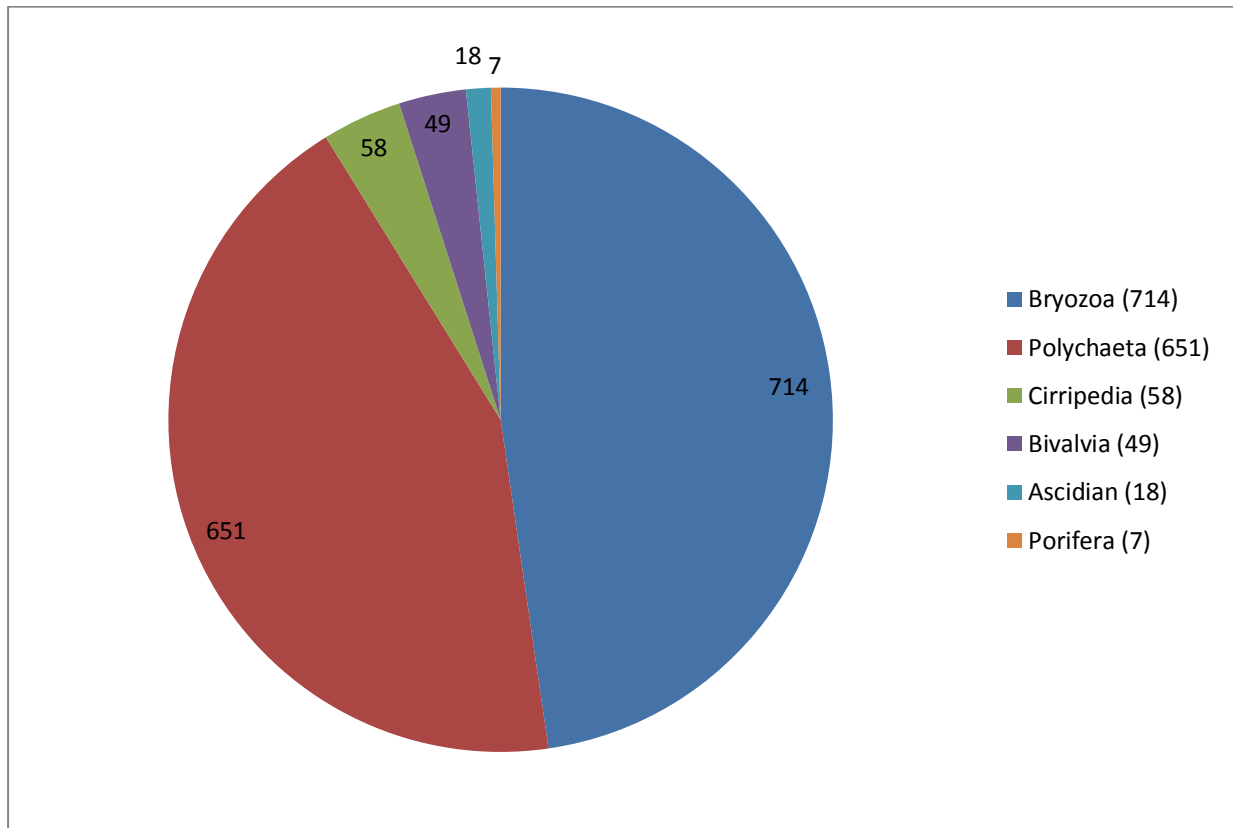
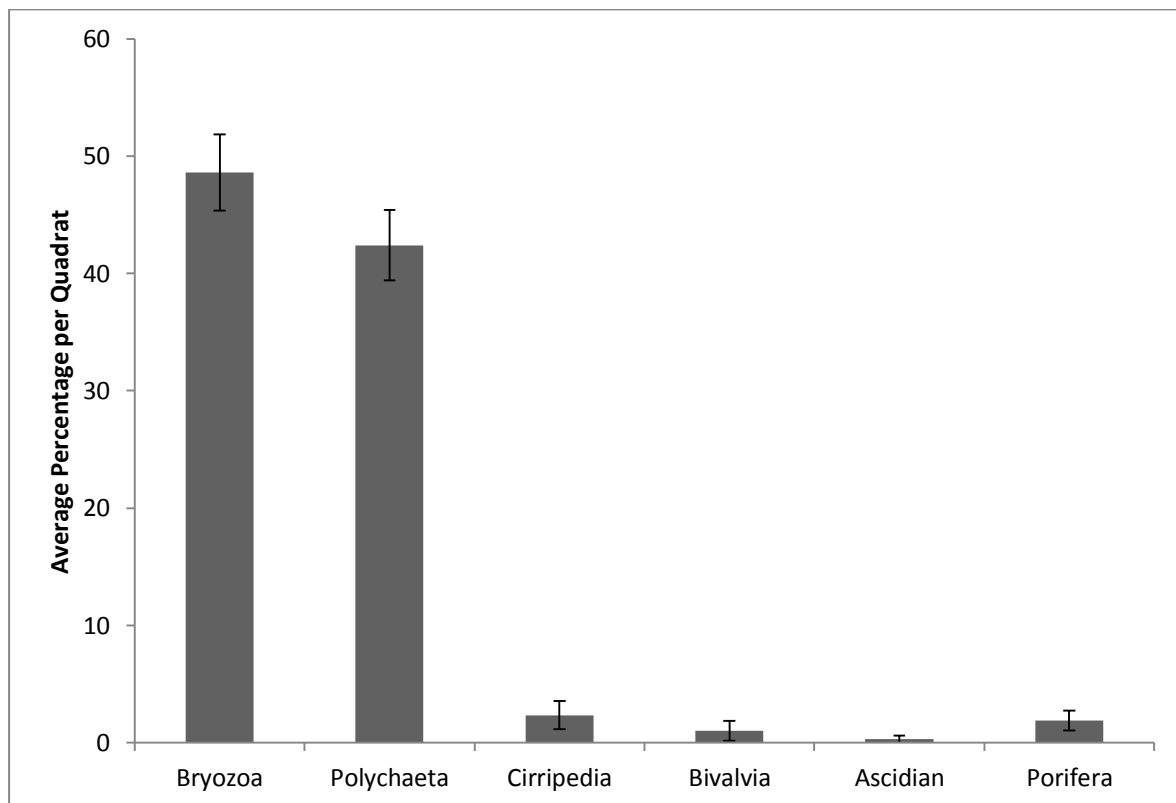


Figure 22: Total number of taxa identified across all plates for the Sea Chest Trails.

A total of 1497 organisms were counted across all plates used in the sea chest trials. Bryozoans made up approximately half (49%) of organisms with 714 individuals or colonies identified (Figure 22). Polychaetes made up a larger percentage of the organisms compared to pilot control plates with 651 (43%) individuals identified. The remaining 8% of organisms were again distributed across Cirripedia (58), Bivalvia (49), Ascidia (18) and Porifera (7). On the control plates bryozoans averaged 48.5% (S.E.  $\pm$  3.25) of organisms observed per quadrat whilst polychaetes averaged 42.4 (S.E.  $\pm$  2.99) (Figure 23).



**Figure 23: Average percentages of organisms of each phylum that settled on control plates after four months of fouling time between April and July 2011 (±S.E.).**

As for Chapter 2, all ecological analysis was undertaken using the control plates of the main trial. Over 95% (S.E. ± 1.44) of organisms survived on the control plates (Figure 24) and there was no significant difference between percentages of organisms across the control plates ( $f = 0.760$ ,  $p = .625$ ). This confirmed consistent and appropriate handling of the settlement plates throughout the experiment. Average taxa abundance was 9.03 (S.E. ± 1.2), no difference in taxa abundance (Figure 25) was observed between control plates ( $f = 0.28$ ,  $p = 0.868$ ). Average taxonomic richness was 2.4 (S.E. ± 1.2) however there was a significant difference in taxonomic richness between control plates ( $F = 2.727$ ,  $p = 0.031$ ) (Figure 26). This difference was primarily due to the statistical difference between plates C5 and C6, these two plates are not significantly different to any other control plate (average difference = 1.250,  $p = 0.021$ ). There was a statistically significant difference in diversity (Shannon  $H'$ loge) (Figure 27) of organisms settling on control settlement plates that reflected the taxonomic richness patterns ( $F = 3.377$ ,  $P = 0.012$ ). There was no overall significant difference in assemblage composition and abundance across the control plates (ANOSIM

Global R = 0.084, sig = 12.4%) (Figure 28). There was no significant difference between assemblages with three months of biofouling and assemblages after 4 months of biofouling (Figure 29).

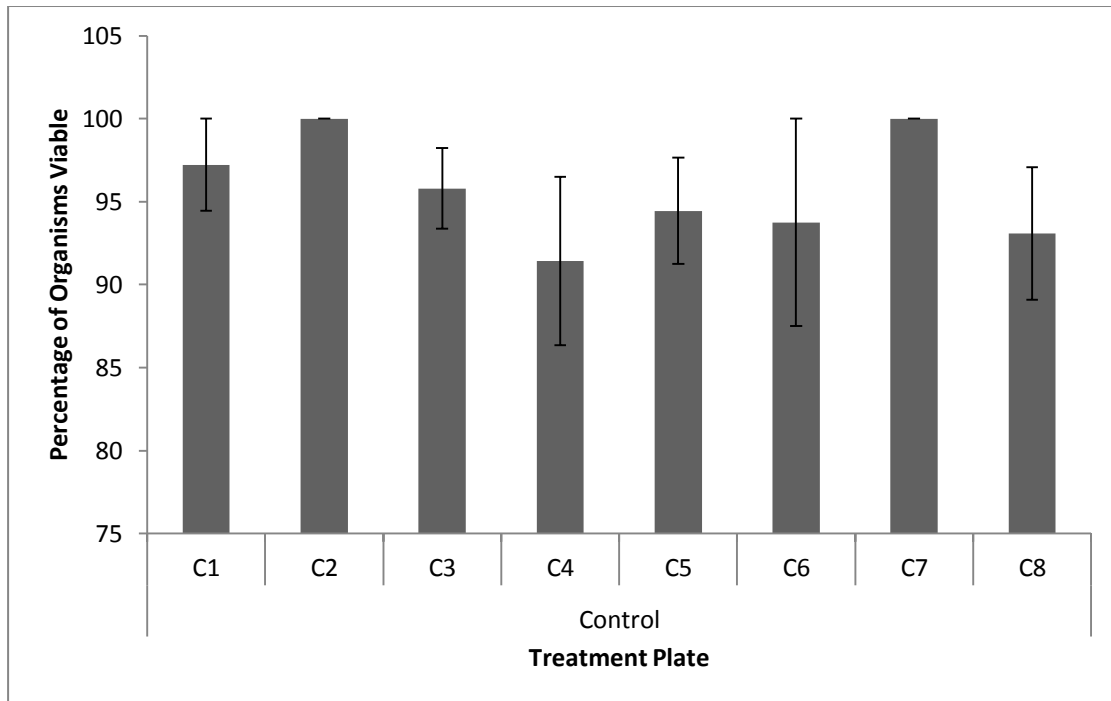


Figure 24: Percentage organism survival across control plates (±S.E.).

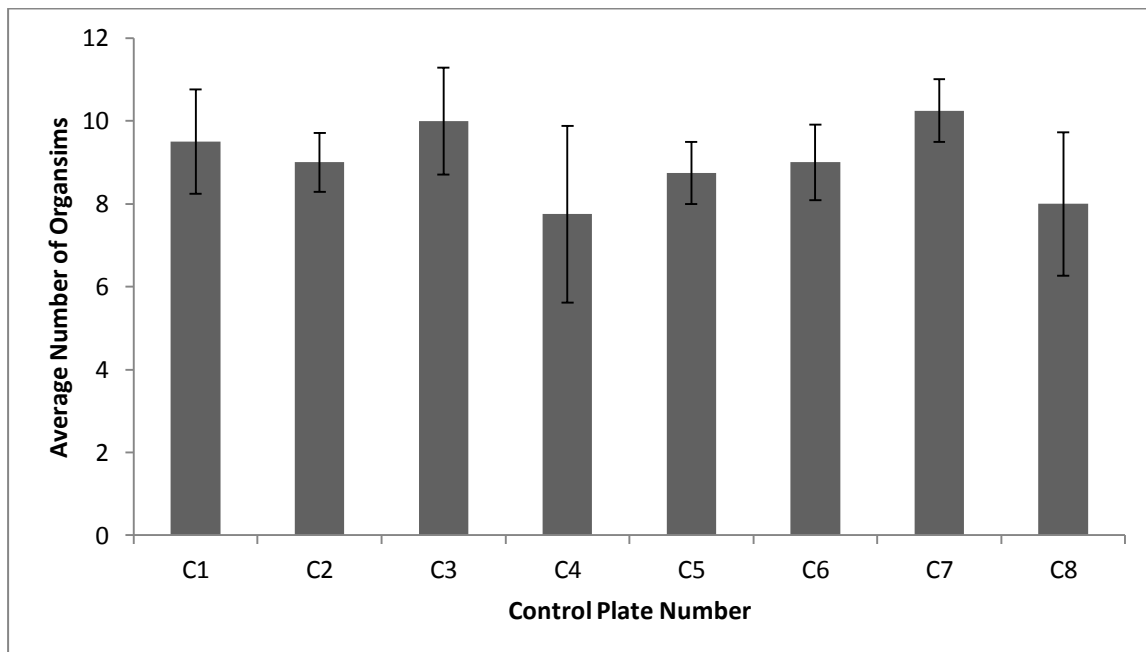


Figure 25: Average total organism abundance across control plates for main trial (±S.E.).

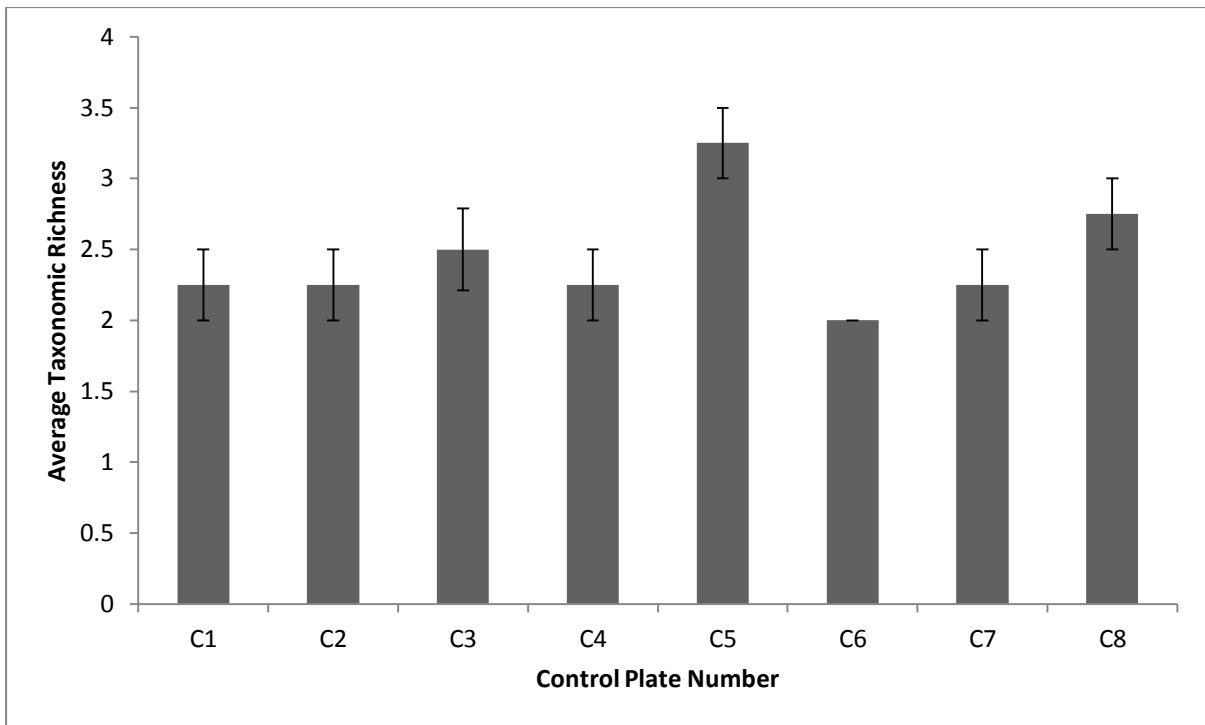


Figure 26: Average taxonomic richness of quadrates on control plates (±S.E.).

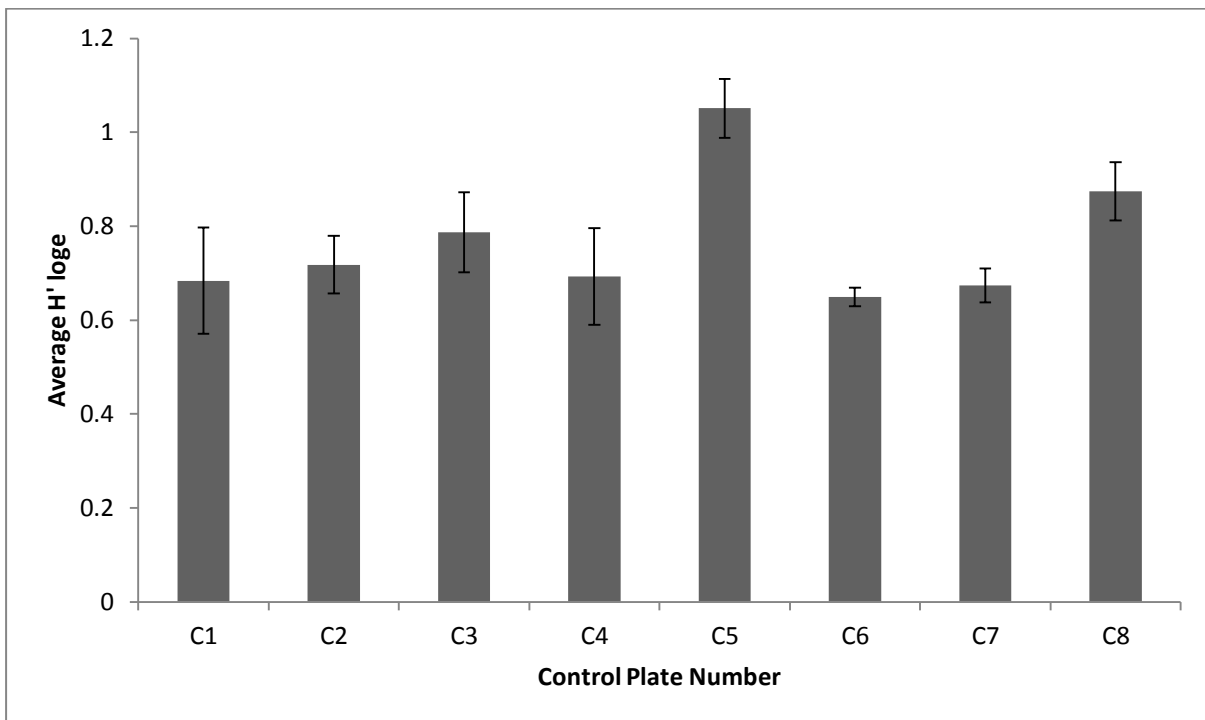


Figure 27: Average Shannon H' loge diversity measure of quadrates on control plates for main trial (±S.E.).



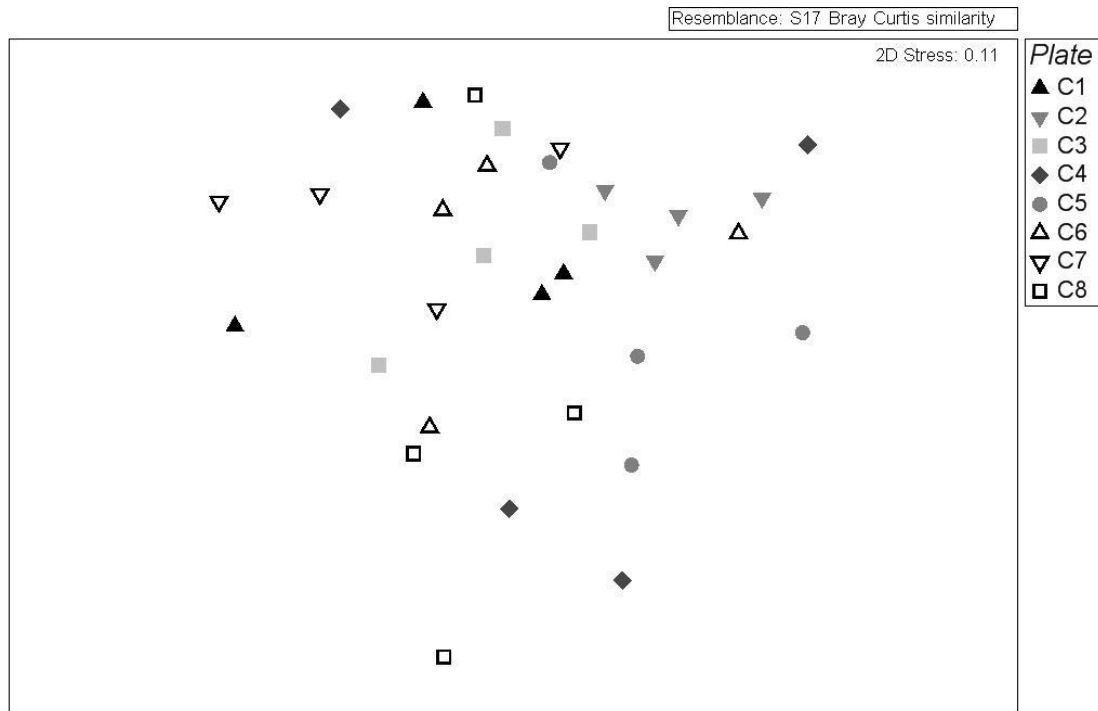


Figure 28: Multidimensional scaling ordination of assemblages on control settlement plates of sea chest trial. No transformation applied to data and the comparison represents both composition and relative abundance of assemblages.

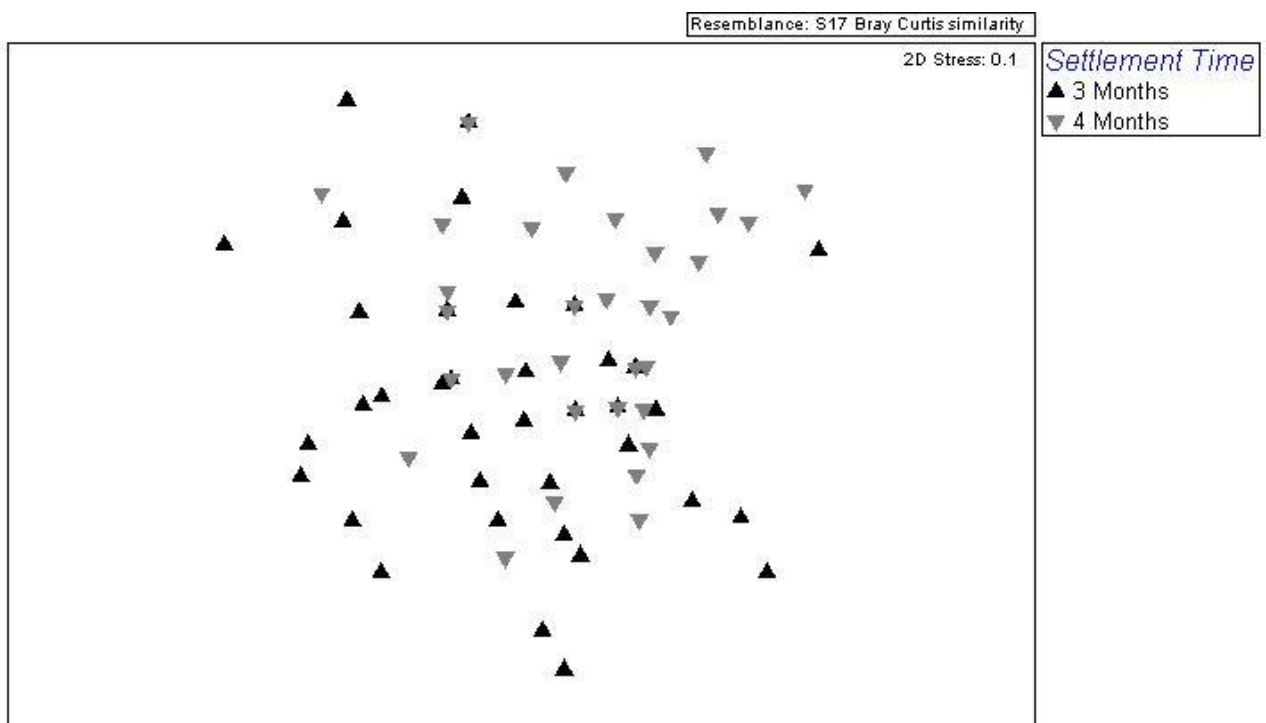


Figure 29: Multidimensional scaling ordination of assemblages on control settlement plates of sea chest trial compared to the assemblages on control plates of the temperature and time pilot trial.

## Temperature Effects on Viability

As was observed in the time and temperature pilot trials, 100% mortality across biofouling on settlement plates was observed. In contrast, there was no significant difference between the 40°C treatments and the controls for mussels (Figure 30) ( $f = 3.000$ ,  $p = 0.114$ ). Both 60°C and 70°C treatments significantly affected mussel mortality ( $f = 13.102$ ,  $p < 0.0001$ ), showing 100% mortality. It appears that hot water reached the corners of the sea chest whilst the top and middle of the front facing panels did not reach the maximum temperature during the two 40°C treatments (Figure 31). There was only 2 replicates of this data, as such no multiple comparison tests were conducted.

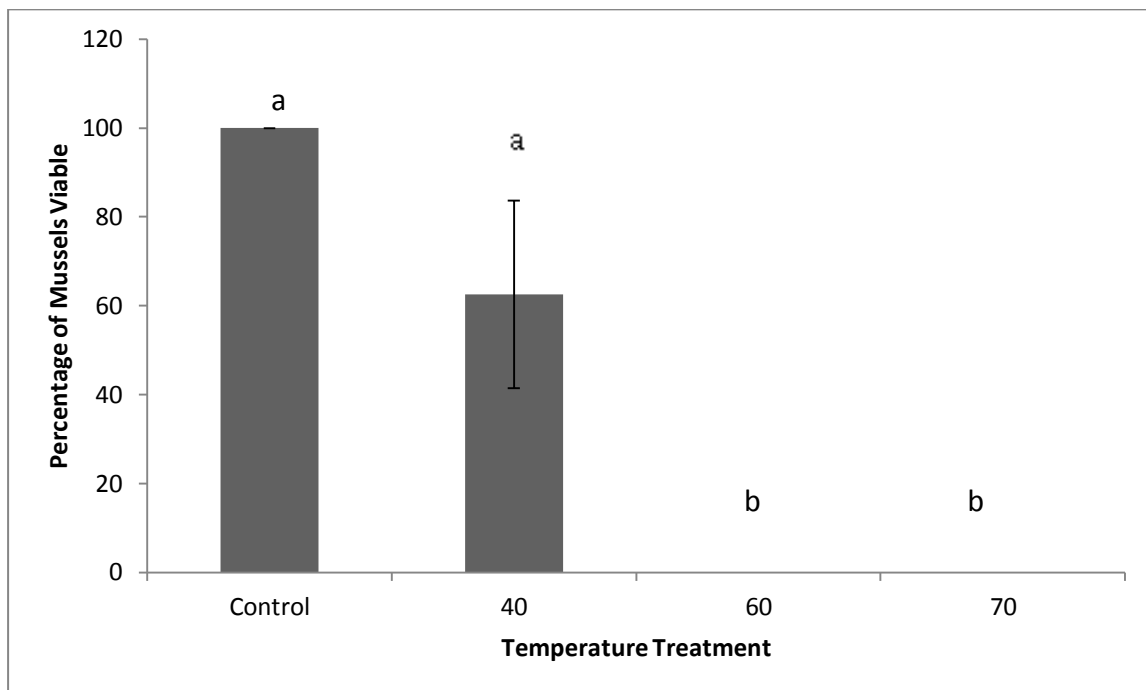


Figure 30: Percentage viability of mussels placed within the sea chest and exposed to temperatures of 40, 60 and 70 degrees for 30, 15 and 10 minutes respectively ( $\pm$ S.E.).

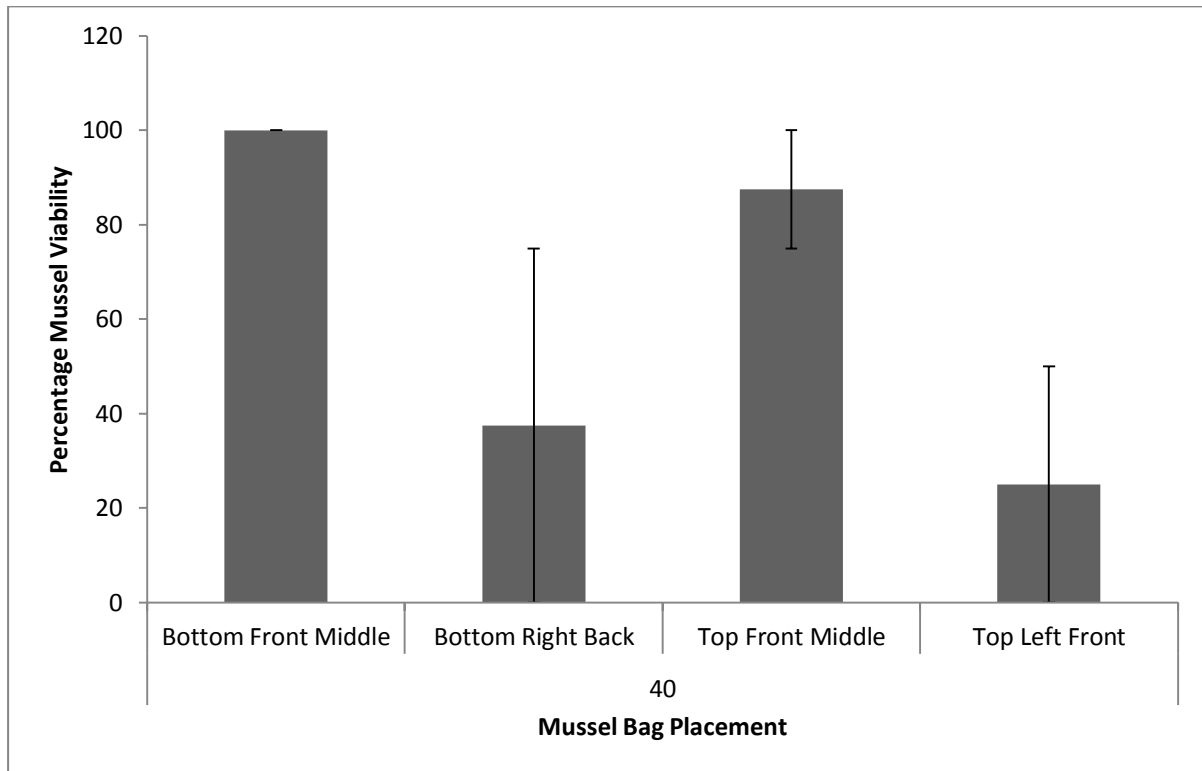


Figure 31: Percentage mussel viability by placement within sea chest at 40°C ( $\pm$ S.E.). There is a trend towards the corners (Bottom Right Back and Top Left Front) experiencing higher temperatures than the front panel of the sea chest.

## Temperature Variability

Temperature varied throughout the sea chest in all trials. The maximum temperature initial run showed a difference of 12.5°C after maximum temperature was reached on the live reading from the HST unit (Figure 32). The differences in temperature experienced throughout the sea chest declined rapidly during the 40°C treatments, with only a 4°C difference experienced. Even at the lowest recorded temperature of 37.5°C there was a 100% mortality rate at the effective exposure time of 15 minutes on the settlement plate.

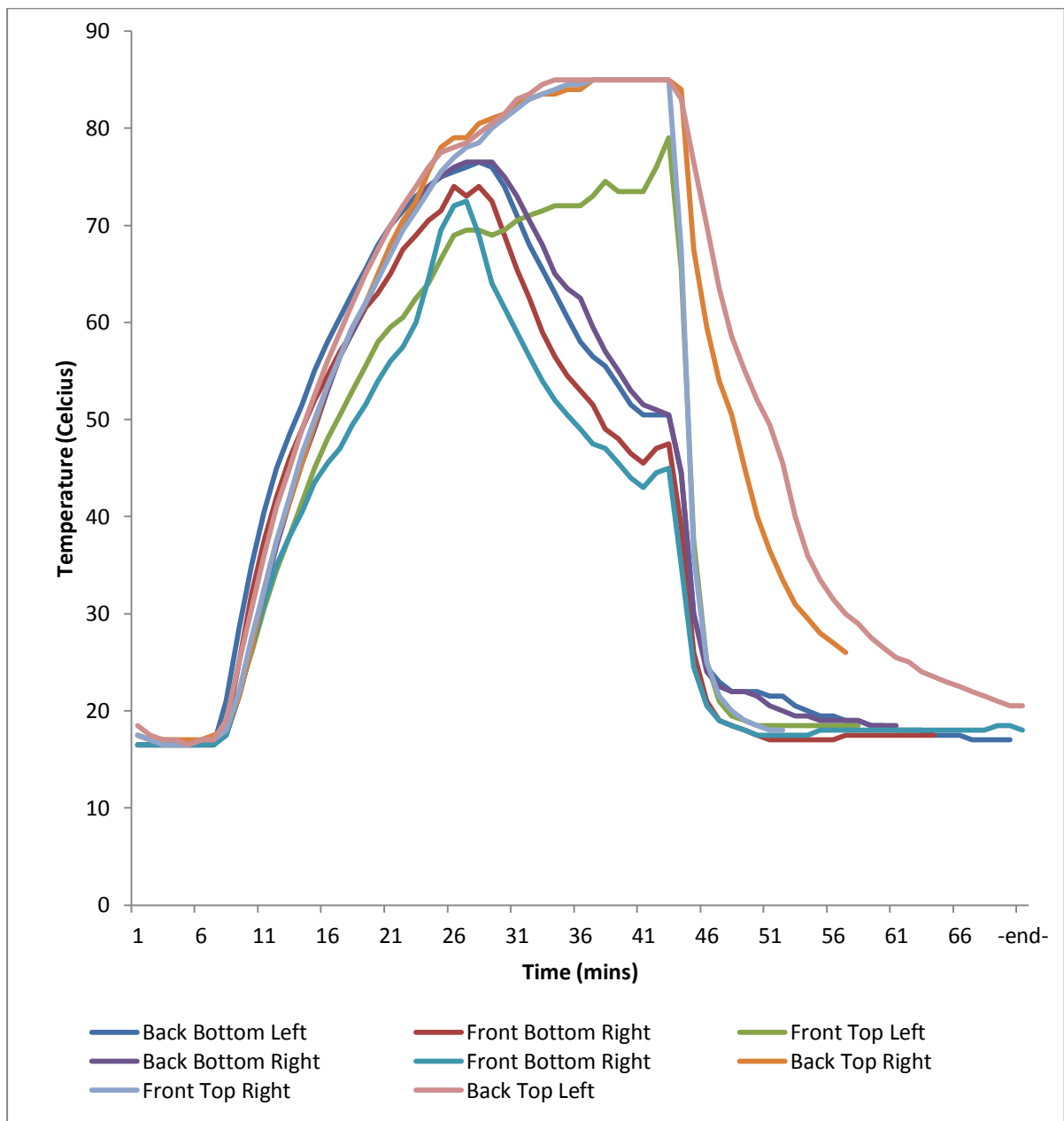


Figure 32: Temperature change in each corner of the sea chest during initial trial.

## **Chapter 4: Discussion**

This study found that a suite of taxa that fouled hard surfaces in shaded environments did not survive exposure to 40°C, and that these conditions were effectively applied in a mock sea chest using hot water treatment technology (HST). The findings establish for the first time that cryptic niche areas of ship hulls can potentially be effectively managed with existing technology and contribute to the preventative strategies of national and international marine pest management systems.

### **Ecological Findings**

There was no overall significant difference in assemblage composition and abundance across the control plates for the temperature and time efficiency trials and the sea chest trials. This tells us that all mortality differences on treated plates are as a direct result of the hot water treatments and treatments within the mock sea chest. The figures 20, 21, 22, 23 and 24 show that plate fouling was relatively uniform across the control (and by extension treatment) plates, with only one plate (C6) showing any statistical difference. 30°C water does not have an effect on non-algal temperate biofouling organisms found in Port Kembla harbour (Figure 14). The plot (Figure 14) also confirms that handling and transportation played no role in changing the assemblages observed on the plates. Comparison of assemblages found on control plates from the temperature and time pilot trail described in Chapter 2 and the control plates of the main mock sea chest trial described in Chapter 3 (Figure 25) found no significant difference in assemblage structure. From this we can infer that non-algal temperate fouling assemblages found in Port Kembla harbour do not significantly change from 3 to 4 months.

Assemblages around marinas have been shown to differ to controls placed in less disturbed environments (Glasby, 1997, Turner et al., 1997). Turner et al. (1997) translocated established assemblages growing on settlement plates from a control location to sites

around marinas. The plates at sites closest to the marinas were found to diverge from the control location. The most recognisable change was the loss of cover by abundant and spatially dominant solitary ascidians; the plates then exhibited an increase in space availability, and a small increase in the cover of sponges, hydroids, bryozoans and colonial ascidians. This differs in assemblage from Glasby (1997) as Turner's (1997) was conducted in New Zealand, assemblage difference is expected, however the observations of an increase in free space, sponges and bryozoans does coincide. It was also observed that assemblage difference coincided with difference in the concentration of heavy metals in suspended sediments (Turner et al., 1997). Port Kembla in the past has been linked with fewer bryozoan species *Bugula nertina* and *Tricellaria porteri* due to increased levels of heavy metals. The development of the polychaete *Galeolaria caespitosa* and the bryozoan *Watersipora arcuata* (an invasive species) were not affected greatly (Moran and Grant, 1993). These species were observed during the survival assessment of both trials however they were not quantified. In an effort to increase the sample sizes more plates had to be analysed, the speed at which the plates had to be analysed to ensure no organism death whilst in aquaria mean the ability to identify organisms to a higher taxonomic resolution was sacrificed. As a result of the added disturbance due to the plates being located on a working jetty and subject to higher heavy metal concentrations the assemblages described in this study may not be indicative of natural fouling assemblages outside of Port Kembla harbour.

Similar to other studies, the assemblages observed fouling on shaded settlement plates were primarily comprised of bryozoans and polychaetes. Glasby (1999) found that free space, number of organisms, polychaetes, sponges, barnacles and hydroids all increased in number in shaded areas. Distance from the sea floor has also been found to interact with shading to have a significant effect on fouling assemblages (Glasby, 1999). The closer the settlement plate was to the sea floor the less light available to the plate, in turn less algae present on the settlement plate. Glasby (1999) saw dramatic decreases in algae numbers in shaded areas near and far from the sea floor when compared to control (light available) areas. Sea chests are completely dark areas as a result no algal species grow within sea chests. The complete lack of algae on settlement plates in this study increases the likelihood

of the assemblages observed being representative of fouling communities inside sea chests of ships in Port Kembla Harbour.

The taxa subject to treatment in this study are largely representative of taxa previously observed inside sea chests. Coutts and Dodgshun (2007) observed a large number of Mytilidae (marine mussels) within sea chests during a survey conducted in New Zealand. Large numbers of *Mytilus galloprovincialis* were observed within the sea chest of a South African Antarctic research supply vessel in 2006 (Lee and Chown, 2007). Other sessile and sedentary taxa observed in sea chests were Porifera, Cnidaria, Bivalvia, Bryozoa, Serpulid and Spirorbid polychaets, Balanidae (Barnacles) and Ascidia. With the exception of Cnidaria all of the taxa observed to settle within sea chests in Coutts and Dodgshun (2007) were represented during the course of these trials. Considering this, the taxa represented on the settlement plates in this study would likely be indicative of taxa settling within the sea chests of maritime vessels in Port Kembla Harbour.

## **Heated Water Applications**

The heated seawater technology tested in this thesis has the ability to achieve and maintain a temperature for the required duration to ensure 100% mortality of biofouling organisms. The temperature and time exposure pilot trials (Chapter 2) in this study established that 3-month-old biofouling was susceptible to treatment of 40°C. This is consistent with Rajagopal (1995) and the observation that a temperature of 39°C was able to deliver 100% mortality of the bivalve *Perna viridis* after 73 minutes. Rajagopal (2005) also observed that *Crassostrea gigas* experiences 100% mortality after 62 minutes at 42°C. The Rajagopal (2005) study only examined oysters larger than 10mm and had no estimates of age. No bivalves were able to grow to that size on the settlement plates in this study, as such it is expected that the assemblages in this study were younger than the organisms tested in the Rajagopal study and thus it was expected that the fouling organisms would expire at lower temperatures and times. However, the results show that 30°C seawater treatments do not significantly affect the mortality of fouling organisms.

Building on these findings, the mock sea chest trials also confirm the susceptibility of tertiary biofouling to heated sea water treatments. *Mytilus edulis* and *Trichomya hirusta* both showed 100% mortality after treatments of 60 and 70°C. This supports previous mussel thermal tolerance research which showed that 30 mins at 55°C was sufficient to ensure 0% attachment from the mussel *Perna canaliculus* (Forrest and Blakemore, 2006). 40°C seawater treatments were not enough to significantly affect the viability of tertiary fouling organisms. This study supports findings by Rajagopal (2005) who found that the *C. gigas* (a species capable of surviving in tropical and temperate climates) at 10.7±1.3mm was able to survive at temperatures of 40°C and 41°C for over an hour. The findings of the mock sea chest trial also concur with Morse (2009) who found that heated water treatments greater than 60°C for 10 seconds or 80°C for 5 seconds were able to achieve 100% mortality in the Zebra Mussel, *Dreissena polymorpha*. These treatments were not mimicked in this study as in the Morse study involved aerial exposure of the mussel the heated spray treatment, not immersion. Longer time periods of complete hot water immersion were tested.

The addition of chlorine or oxidants to heated water was suggested as a possible way to treat for *D. polymorpha* (Harrington et al., 1997). The idea being that the addition of oxidants would mean that the temperature of the water would not need to be raised as high or maintained for as long to ensure 100% mortality. When combined the use of heat and oxidants decreased the time to 95% mortality by more than 95% at 30°C (Harrington et al., 1997). At 36°C however, the differences between the combined treatment strategies over heat alone were minimal. Considering the risks the addition of chlorine may pose to the local environment and that the use of 40°C does significantly slow down the HST unit or slow its output the addition of oxidants for the treatment of biofouling is unnecessary.

Optimum temperature treatments and times identified in this study were 40°C at an exposure time of 15 minutes for all secondary biofouling and 60 and 70 °C for 10 minutes for tertiary biofouling. Thermal shock has been shown to induce mortality of many marine species (Rajagopal et al., 1995, Thiyagarajan et al., 2000, Rajagopal et al., 2005, Morse, 2009), however at 40°C, exposure for longer than 15 minutes may be required to avoid propagule or organism escape. It was observed anecdotally that at 40°C, treatments polychaetes may have tried to escape. There was no evidence of escape at higher



temperatures (60°C and 70°C). The slow heating times in a sea chest will require the sea chest to be sealed to prevent propagule release or mobile adult organism escape. When applying hot water treatments to other parts of vessel higher temperatures (greater than 60°C) will have to be used to ensure the biofouling organism has expired before it has a chance to escape the hot water or spawn. This would apply to hot water treatments on surfaces where sealing the water would be impossible, such as other niche areas of a vessel, including the rudder hinge, propeller, bilge keel and bow thrusters.

## **Marine Pest Applications**

The biofouling taxonomic groups represented in this survey represent many marine pest species that are known to have invaded new areas. The invasive bryozoans *Watersipora arcuata* and *Bugula nertina* were observed fouling settlement plates throughout this study. *W. arcuata*, a Mexican-Pacific native has been spread to Australia and Hawaii due to its ability to rapidly colonize surfaces of degraded antifouling paint on ship hulls (Mackie et al., 2006 and references there in). *Bugula neritina*, an upright-branching bryozoan, was considered cosmopolitan at the time of first taxonomic identification; however further genetic and bacterial-symbiont diversity analysis has shown that the taxon is the three cryptic species. The Type S species has found to be widespread throughout Australia, Hong Kong, Curacao, Hawaii and England (Mackie et al., 2006 and references there in). A famous example of an invasive polychaete is the European fanworm, *Sabella spallanzanii*, introduced into Port Phillip Bay, Victoria in the late 1990s it is now a prominent part of most benthic communities. A 1998 dive survey showed *S. spallanzanii* has extended its range to cover the entire 2000 km<sup>2</sup> embayment and had invaded most sub tidal habitats (Currie et al., 2000). The primary methods of transport for *S. spallanzanii* is biofouling on ships and inside internal seawater systems (NSPMMPI, 2009), and in sight of its current abundance, high fecundity and long spawning periods, there is a high risk of future expansions (Currie et al., 2000). Although this species was not found on settlements plates in this study, it is of similar size and habitat to the biofouling polychaets identified in this study as well the temperate distribution of invasion, these factors indicate that at least the biofouling life stages would of *S. spallanzanii* be susceptible to temperatures of 40°C and above, however

further testing on adult stages of the polychaete would be required to establish species specific susceptibility heated sea water treatments. Future work might consider testing temperatures between 30°C and 40°C to find the minimum temperature and time exposure required to eliminate primary and secondary biofouling, which might benefit the efficiency of application in commercial practice.

Based on the results of this study, HST at elevated temperatures of 60 or 70°C for a period of 10 minutes is capable of inducing 100% mussel mortality inside sea chests. HST is a potential tool for mitigating the transfer of tertiary biofouling NIMS via sea chests. A mussel species known to inhabit sea chests is the Mediterranean mussel, *Mytilus galloprovincialis*. Native to the Black, Adriatic and Mediterranean Sea this species has successfully established itself widely around the world in temperate regions where there are large shipping ports (Branch and Nina Steffani, 2004). One particularly concerning report of *M. galloprovincialis* biofouling was its presence in large numbers within the sea chests of the South African National Antarctic Programme supply vessel, the SA 'Agulhas' (Lee and Chown, 2007). This is particularly worrying as the size of the mussels indicated that they had survived transportation to the Antarctic region on multiple occasions. Whilst the mussel hasn't been recorded in the Antarctic region there is the very real possibility that this species could inhabit areas such as Marion Island and Gough Island (Lee and Chown, 2007). To test heated seawater's ability to treat tertiary biofouling the species *Mytilus edulis* was used. *M. edulis* and *M. galloprovincialis* both being from the same genus and native to similar climates it is expected that the time and temperature treatments used to treat *M. edulis* will also be effective to treat the biosecurity risks posed by *M. galloprovincialis*.

## **Hull Surface Treatment Viability**

An observation made during this study was that at higher temperatures there is a fairly large variability of temperatures experienced at different places within the sea chest. Considering the maximum flow rate (38 litres per minute at time of trials) at which the HST unit can operate and the size of the mock sea chest it is predicted that larger sea chests will experience even greater variability, compensations will need to be made during for the

technology's application. At the time of writing a new two-stage pump has been added to the HST unit delivering 120 litres per minute at a temperature up to 98°C. Similar trials will need to be undertaken with a larger mock sea chest (e.g. 2 x 1 x 1m) or by placing temperature loggers in a real world and larger sea chest during a HST application. This would inform HST's application to larger vessels as well as help determine a relationship between sea chest size and temperature variability. Rajagopal (2005) was able to induce a higher thermal tolerance in *C. gigas* by exposing the oysters to 1 hour treatments of 37 and 39°C 14 days before treating the oysters. For example at 40°C oysters exposed after previous thermal shock and oysters exposed without previous thermal shock took 156 and 123 min, respectively to achieve 100% mortality. This could have a dramatic effect on the application of HST. The temperature variability within the sea chest is such that if a tertiary fouling organism is exposed to temperatures lower than 40°C it could mean the next time it is treated at a higher temperature it may have acquired a thermal tolerance high enough to survive the new treatment. Considering this and the temperature variability experienced inside the sea chest means higher temperatures (60 or 70°C) should be used to treat tertiary biofouling.

During the survivorship assessment it was observed that when some polychaetes' tubes were disturbed in anyway the worm would release propagules. Mobile amphipods were also observed on plates during the survival assessment phase of the time and temperature trials. HST has a specific advantage when it comes to managing marine pest incursions resulting from sea chest transport, which is the applicator's ability to form a soft seal around the grate of the sea chest. It has been observed that any active treatment of sea chest will have to account for both mobile organisms and propagule release (Coutts and Dodgshun, 2007, Coutts et al., 2003). The seal formed means that mobile organisms and propagules will not escape during treatment when the organism is first disturbed. It was also observed at during the survivorship assessment of 40°C plates that many polychaete tubes were empty or had the remains of the worm caught on the points at the entrance of the tube, this could be indicative of organisms trying to escape the heated water. Due to temperature variability within the sea chest at higher temperatures some organisms may not experience the maximum temperature within the sea chest. This is particularly problematic when trying to treat adult mobile organisms, because heating is not uniform, mobile taxa could seek refuge

in cooler parts of the sea chest, survive the treatment then escape afterwards. As a result, not only will the biofouling have to be isolated during treatment but higher temperatures maintained (60 or 70°C) to ensure mobile taxa are treated to a lethal exposure to heated seawater.

The power of the results in Chapter 3 make up for the pseudo-replicative effects of using multiple plates within the sea chests as replicates each temperature treatment once. Future studies however may use more than one run of each temperature and time regime. The temperate bryozoan dominated assemblages found in Port Kembla harbour during the course of this study are not indicative of biofouling assemblages found in other tropical or other temperate zones (Turner et al., 1997, Satheesh and Wesley, 2011). Testing the temperature tolerances of different biofouling assemblages will be required to determine this technology's efficacy for use around the world. Key to this may be the thermal tolerances of organisms in tropical regions that routinely experience warmer temperatures, in this situation 40°C may not be high enough to induce 100% mortality of biofouling organisms (Rajagopal, 2005). The ambient temperature of the water which the HST unit will have to heat in tropical conditions, the temperature variability within the sea chest will be affected. The higher ambient water temperature will mean that the HST unit will be able to heat the water faster and deliver higher temperatures more efficiently to the sea chest as less heat will be drawn from the hose, applicator or sea chest to the outside water. Ideally the trials conducted in this study should be conducted in tropical waters.

The three and four month non algal temperate epibiotic assemblages recorded in this study may not be indicative of three and four month from other times of the year. It has been known for the better part of a century that on the New South Wales coastline organisms experience settlement and different rates throughout the year (Allen and Wood, 1950). There is the possibility that the different fouling assemblages from different times of the year will have different thermal tolerances. Due to time constraints of an honours project this study was only able to assess winter fouling assemblages (1 April to 4 August 2011). Future studies should also test HST's ability to effectively treat 3-4 month assemblages from the three other seasons of the year (Spring Aug – Nov, Summer Nov – Feb and Autumn Feb-Apr).

Biofouling may show a faster rate of recruitment after HST treatment. Fouling, or the remains of, on a vessel following treatment may also provide a refuge for newly settling fouling taxa. The invasive bryozoan *Watersipora subtorquata* is known to act as a foundation species for fouling assemblages colonising areas treated with anti-fouling paint (Floerl et al., 2004). During this survey the hard remains of bryozoans and ascidians as well as the calcareous tubes of polychaetes were present on settling plates long after treatment. Whilst the extreme water flow experienced within sea chests may mean some of the remains will be removed, areas of low water flow may show higher rates of recruitment after HST treatment. This means that although HST has the ability to treat tertiary biofouling within sea chests it would be most effective as part of continual antifouling maintenance regime. Three months was observed to accumulate a 15 - 40 % cover of biofouling organisms on tin free copper based antifouling paints (Jelic-Mrcelic et al., 2006). Notable animal taxa included Serpulid polychaetes, Encrusting Bryozoans and Barnacles. After just 6 months Mussels began to dominate the assemblages. Once secondary biofouling has initiated within the sea chest treatment HST treatment will have to become more regular to mitigate the biosecurity risks posed by higher recruitment rates. The complete prevention of biofouling is the ultimate goal of all antifouling strategies. To ensure a biofouling free sea chest regular treatments at regular intervals less than 3 months (e.g., monthly) after dry-docking is required.

Ideally vessels would be regularly treated (e.g., monthly), thus preventing tertiary biofouling. However, HST could be used to treat maritime vessels already expressing tertiary biofouling before they enter a port. The treatment before the ship enters port would mean that any biosecurity risks inside the sea chest will be accounted for before the vessel reaches a shallow environment suitable for organism escape or settlement. The biofouling remains within the sea chest may then increase the recruitment of more fouling organisms (Floerl et al., 2004), however any in-water cleaning of an antifouling painted surface is illegal in Australian waters (NIMPCG, 2009). Sea chests are occasionally allowed to be defouled in-water however this is at the discretion of the appropriate state or territory regulator. Hopkins (2010) described 3 situations where the biosecurity risks of in-water defouling are likely to be low: 1) The defouling method retains close to 100% of defouled material; 2)

Biofouling has been previously treated and is no longer viable; and 3) Defouling is carried out over sub-optimal habitat (e.g. open ocean) to minimise survivorship. If the vessel's sea chests are treated with HST before entering a port then the biological risks associated with in-water fouling would be insignificant so long as the antifouling paint coat remains undamaged. Currently it is not standard practice for vessel owners/companies to examine their vessel's fouling levels to determine the NIMS risk. Surface observations of vessel fouling are not a useful predictor of sub-surface fouling (Hopkins, 2010). As such, a precautionary approach should be taken and all vessels that have not received a defouling treatment within the previous three months should be treated with HST before they enter port.

In an ideal world the HST applicator will be applied to the side of the hull and when, during the course of its hull application, it comes across a sea chest the applicator will be placed over the grill of the sea chest and initiated (Figure 4). For hull treatment the HST unit is usually heating water to temperatures of  $>70^{\circ}\text{C}$ . For the course of its application to the sea chest, this temperature should be maintained in the boiler whilst the sea chest should be heated to a minimum of  $60^{\circ}\text{C}$  for 10 minutes to ensure total biofouling mortality. The HST applicator is not applicable to other vessel niche areas such as dry docking support strips, rudder hinge, propeller, bilge keel and bow thrusters. This reinforces that notion that HST is just one tool available for the treatment of vessel biofouling. To minimise the biosecurity risks associated with vessel biofouling HST should be used in conjunction with properly applied and maintained anti-fouling paint, and regular dry-dockings. HST is a viable tool for the first strategy of the National System for the Prevention and Management of Marine Pest Incursions – prevention of marine pest incursions.

## **Conclusion and Future Research**

In conclusion, the application of  $40^{\circ}\text{C}$  sea water that was maintained for 15 minutes was shown to be an effective treatment either by immersion or the use of HST technology to eliminate all secondary biofouling organisms in this study. For tertiary biofouling higher temperatures of at  $60^{\circ}\text{C}$  for 10 minutes are required within the sea chest to ensure all

tertiary biofouling and adult mobile organisms have expired within the sea chest. The temperate non-algal epibiotic assemblages present on settlement plates used for the course of this study are likely to be reflective of the biofouling within commercial vessels of Port Kembla Harbour. The taxa exposed in this treatment were NIMS themselves, or representative of taxa that have currently invaded marine habitats in Australia or around the world. HST has the ability to isolate the sea chest and ensure all biofouling is treated before propagules or mobile organisms have a chance to escape. These results show that HST is a viable option for treating the biosecurity risks associated with biofouling within sea chests. There was a significant temperature variability observed throughout the sea chest during treatment HST should be trialled on larger sea chests. Assemblages and thermal tolerances of organisms are different in other regions and climates of the world ideally this study should be repeated in tropical waters. Currently HST cannot treat other vessel niche areas, as such HST should be used in association other antifouling and defouling measures and maritime regulatory practices to prevent future NIMS incursions.

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## Appendices

### Appendix 1: Guidelines used to assess viability of fouling taxa on treated settling plates.

	Indicators for live and viable individuals/colonies	Indicators for non-viability of individuals/colonies
Barnacles	<p>Structure: All shell plates present and intact, opercular plates present (acorn barnacles only – gooseneck barnacles have no opercular plates).</p> <p>Feeding/movement: Feeding structures (cirri) protrude out of the test and perform sweeping feeding movements. Or opercular shells closed by muscular action.</p>	<p>Structure: Shell/opercular plates and/or feeding structures (cirri) broken or missing.</p> <p>Feeding/movement: Feeding structures visible but motionless and slack and/or no reaction when poked.</p>
Bivalves	<p>Feeding/movement: Shells may be locked by muscular action (i.e. this bivalve lives). Shells may also be open (feeding), exposing mantle tissue and siphons (or gaps in mantle), but will close when poked (reaction).</p> <p>Structure: Both shells present and intact.</p>	<p>Structure: One shell missing or one/both shells cracked or fragmented.</p> <p>Feeding/movement: Shells open but no reaction to touch.</p>
Encrusting bryozoans	<p>Structure: Colony/fragment contains several intact zooids (check for animal inside against light).</p> <p>Feeding/movement: Filtering apparatus (lophophore) protrude through opening in zooid.</p>	<p>Structure: All zooids damaged/smashed, no soft tissues visible. And/or: all colonies dried out, loss of all moisture. And/or loss of pigmentation.</p> <p>Feeding/movement: Zooids' soft tissues and/or feeding structures may be visible but no movement or reaction to touch.</p>
Erect bryozoans	<p>Structure: Colony/fragment contains several intact zooids (check for animal inside against light).</p> <p>Feeding/movement: Filtering apparatus (lophophore) protrude through opening in zooid.</p>	<p>Structure: All zooids damaged/smashed, no soft tissues visible. And/or: all colonies dried out, loss of all moisture.</p> <p>Feeding/movement: Feeding structures may be visible but no movement or reaction to touch.</p>
Colonial ascidians	<p>Structure: Colony/fragment in reasonable 'shape', moist to the touch (not dried) and not entirely crushed. Several polyps intact.</p> <p>Feeding/movement: Inhalant and/or exhalant siphons open but close when poked.</p>	<p>Structure: Shredded or crushed so that badly damaged. No polyps visible (polyps may have 'popped out' from mechanical pressure on colony). And/or colony dried out, loss of all moisture.</p> <p>Feeding/movement: Siphons open but no reaction to touch.</p>
Solitary ascidians	<p>Structure: Test (body) intact, no holes or gashes, not crushed flat or severely deformed. Moist, not dried.</p> <p>Feeding/movement: Inhalant and/or exhalant siphons open but close when poked (reaction).</p>	<p>Structure: Test badly damaged, crushed or deformed. Branchial basket exposed and/or damaged, guts hanging out. And/or colony dried out, loss of all moisture.</p> <p>Feeding/movement: Siphons open but no reaction to touch.</p>

Hydroids	Structure: Body reasonably intact, feeding polyps (often at distal ends of braches) present. Feeding/movement: Feeding tentacles exposed.	Structure: All polyps damaged/smashed. And/or colony dried out, loss of all moisture. Feeding/movement: Feeding structures may be visible but no movement or reaction to touch.
Tubicolous polychaetes	Structure: Intact (body within tube), not crushed, no holes or gashes. Feeding/movement: Worm retracts into tube when poked (reaction), and/or feeding structures (tentacular crown) visible and moving.	Structure: Tube missing, loss of tentacular crown, body badly crushed or lacerated. And/or dried out, loss of all moisture. Feeding/movement: Feeding structures may be visible but no movement or reaction to touch.
Sponges (assessment of viability very difficult or impossible)	Structure: Fragments retain natural colour, firm texture (don't fall apart). Sponges retain a "fleshy/translucent/shiny" appearance. Look for "translucent" tissue between fibres Feeding/movement: Impossible to observe.	Structure: Colony/fragment faded and bleached, falling apart. Sponge a mass of golden fibres/hair-like structures without "translucent fleshy tissue" between the fibres. And/or colony dried out, loss of all moisture. Usually no chance for survival if removed from water for more than 3 hours.
Macroalgae	Structure: Contain pigment and have natural colour. Dryness often not a good indicator as some species are intertidal. Look out for and preserve reproductive structures. Feeding/movement: n/a	Structure: Badly crushed, fragmented, or faded (loss of pigments). Feeding/movement: n/a
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(Woods et al., 2005)