Ministry for Primary Industries
Manatū Ahu Matua

Literature review: In-water systems to remove or treat biofouling in vessel sea chests and internal pipework

MPI Technical Paper No: 2016-16

Prepared for Animal and Marine Biosecurity Response, Long-term Incursion Management, Surveillance and Incursion Investigation and Biosecurity and Environment Groups

By: Abraham Growcott, Dan Kluza and Eugene Georgiades
Risk Analysis Team: Animals and Aquatic

ISBN No: 978-1-77665-210-5 (online)
ISSN No: 2253-3923 (online)

March 2016

New Zealand Government

Growing and Protecting New Zealand

Disclaimer

While every effort has been made to ensure the information in this publication is accurate, the Ministry for Primary Industries does not accept any responsibility or liability for error of fact, omission, interpretation or opinion that may be present, nor for the consequences of any decisions based on this information.

Requests for further copies should be directed to:

Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries website at http://www.mpi.govt.nz/news-and-resources/publications/

Executive Summary

Vessel biofouling is a major pathway for the introduction and spread of non-indigenous marine species. Sea chests are cavities built into a vessel's hull to help increase the efficiency of pumping seawater into the internal pipework system. Biofouling of the internal surfaces of sea chests is able to occur through the entry of larval stages of sessile species through the sea chest grates. As sea chests are areas that are protected from a constant laminar flow of water they tend to accumulate a higher biomass of organisms than general hull areas.

The International Maritime Organization recommend the use of marine growth protection systems and antifouling paints to prevent the accumulation of biofouling within sea chests and internal pipework. Despite this, some issues associated with the efficacy of these systems have been identified. In addition to the transport of non-indigenous species, the consequences associated with sea chest and internal pipework biofouling include impacts to vessel operational efficiency and crew safety.

Currently, the only way to reactively[1] minimise the biosecurity risks associated with sea chest and internal pipework biofouling is to physically remove it from the vessel in dry-dock. This process, however, is expensive in both time and money and dry-docking facilities in Australia and New Zealand are often in high demand or not equipped to handle large commercial vessels.

In-water systems for reactive use on the biofouling of sea chests and internal pipework are not currently available in New Zealand for border and post-border management of vessel biofouling. This document has identified and reviewed potential in-water systems for reactive removal or treatment[2] of biofouling in sea chests and internal pipework within the following broad categories:

- chemical,
- non-chemical, and
- co-treatments.

Within these categories exist system types that warrant the development of testing frameworks and performance standards. These include: application of oxidising and non-oxidising chemicals, physical removal, thermal treatment, and co-treatments. The following biosecurity risks have been identified and these will inform the development of the testing frameworks and performance standards:

- material may be dislodged from the sea chest grates by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system or equipment used to blank off the sea chests,
- containment associated with the initial application of treatment to achieve the target concentration (where biocides are used),
- containment associated with equipment used to blank off the sea chests,
- efficacy of the system treating or removing the biofouling,
- capture of waste material removed (where removal is undertaken),

[1] *Reactive* in this context relates to in-water systems that are capable of cleaning or treating biofouling that has already settled on a vessel's surface as opposed to preventative systems where the settlement of fouling organisms is prevented.

[2] For the purposes of this document in-water cleaning is defined as the physical removal of biofouling organisms from a surface. In-water treatment is defined as the killing of biofouling organisms in situ.

- filtration of captured waste (where removal is undertaken) and how effectively, and to what minimum particle size, material is removed from the effluent stream, and treatment of effluent (where removal is undertaken, for example, with heat, ultra-violet light or biocides) or discharged to a sewerage system with secondary treatment.

It should be noted that use of these systems carries some residual biosecurity risk that must be managed. Further, to ensure uptake, systems must be efficacious within the constraints of duration of international vessel dockings within New Zealand.

Contents

[3] *Erratum 2019: The authors acknowledge that acetic acid and some acids used as active ingredients of descalers are in fact non-oxidising acids.*

1. Background

This document contributes to the scientific background for approval of in-water cleaning or treatment systems under the Craft Risk Management Standard for Biofouling for Arriving Vessels. The Ministry for Primary Industries (MPI) will consider this document along with other information in determining proposed measures that are practical to implement and align with all applicable legislation, while ensuring the biosecurity risk does not exceed New Zealand's appropriate level of protection.

As a first step, an understanding of current and emerging in-water systems for sea chests and internal pipe-work is required to inform their broad categorisation. The biosecurity risk associated with each broad category will be identified and inform the requirements of any performance standard developed (e.g. organism viability, filtration). This review will also identify the feasibility of each in-water system category and the appropriateness for developing performance standards and testing requirements.

2. Introduction

Sea chests are cavities built into a vessel's hull that house the openings to the internal pipework system. They are positioned below the water line on the side or bottom of the hull (Fig.1). Sea chests help increase the efficiency of pumping seawater on board when the vessel is in motion by providing a motionless reservoir of water for ballast, firefighting and engine cooling (Palermo 1992; Coutts *et al.* 2003). To prevent the entry of large debris, sea chests are protected by grates. These grates are either attached to the hull with bolts or welded on, therefore, the internal area of the sea chest may only be accessible when the vessel is dry-docked (Coutts *et al.* 2003). Even then, the sea chest may only be accessible by cutting into the hull (Justin McDonald pers. comm.). The size, number and complexity of sea chests generally increase with vessel size (Coutts and Dodgshun 2007).

2.1. Biofouling of submerged surfaces

Although a sea chest grate is designed to prevent the entry of large debris, biofouling of the internal surfaces is able to occur through the entry of larval stages of sessile species (e.g. Frey *et al.* 2014). Biofouling is a process by which organisms accumulate on structures immersed in the aquatic environment. The generalised succession of biofouling is as follows:

- once the structure is submerged organic and inorganic molecules are adsorbed onto the exposed surface,
- microbial cells and bacteria attach,
- these organisms exude extrapolymeric substances, forming the characteristic 'slime layer',
- multicellular organisms (e.g. bivalves, macroalgae) settle to form a more structurally complex community, and
- as the complexity of biofouling increases, habitat is created for mobile organisms (crabs, fish, isopods etc.) (Lehaitre and Compere 2005; Bell *et al.* 2011).

Recent experimental work has shown that biofouling may not occur in this generalised sequence. For example, the spores of *Ulva* spp. have been observed settling on a submerged surface that had been 'cleaned' before exposure (Joint *et al.* 2000). Instead, the settlement and establishment of biofouling is a process where all the above stages may occur in parallel and the outcome is determined by dynamic and complex interactions between abiotic (e.g. surface characteristics, water chemistry, water flow, depth) and biotic (e.g. species abundance

and composition, inter- and intra- species competition) factors (Lehaitre and Compere 2005; Bell *et al.* 2011).

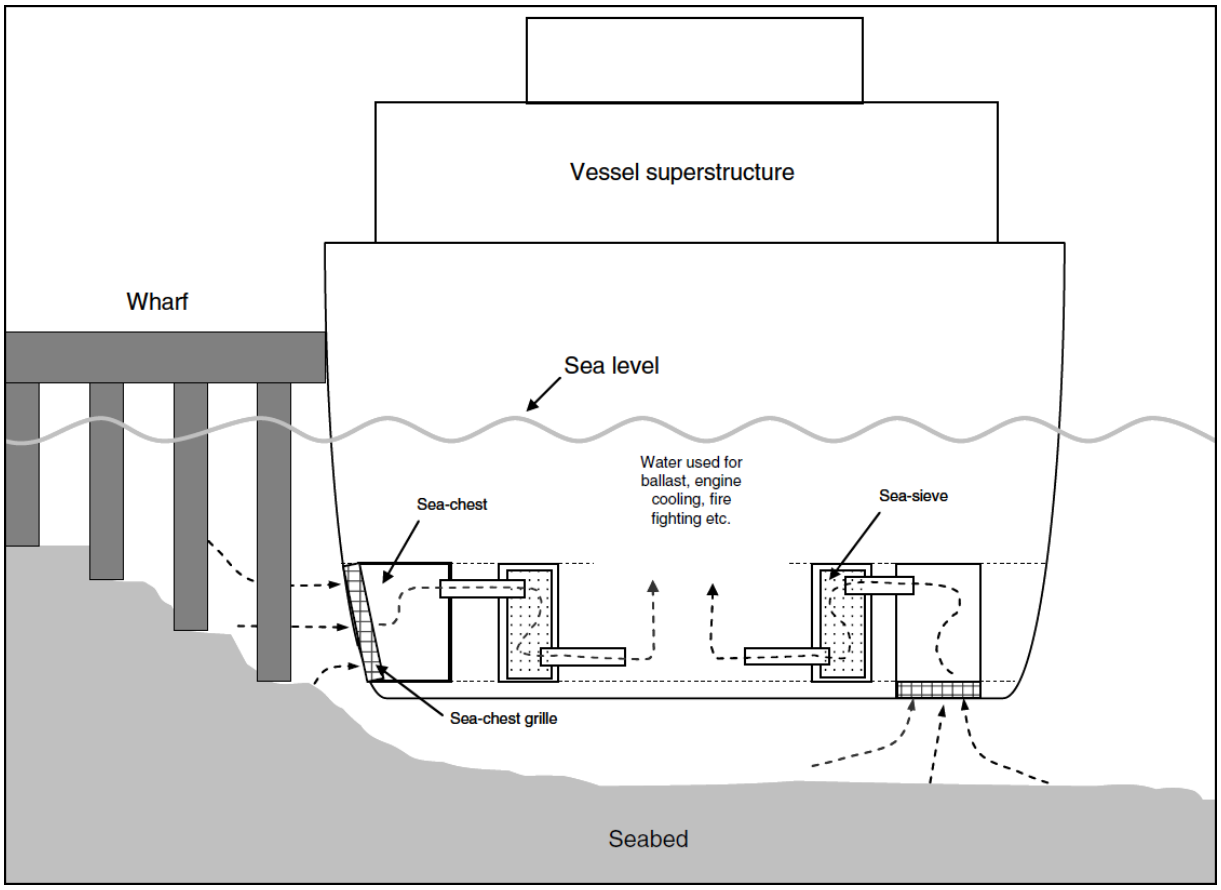

Figure 1. Diagram of vessel sea chests (from Coutts and Dodgshun 2007).

2.2. Consequences of sea chest and internal pipework biofouling

Biofouling of sea chests and internal pipework may reduce a vessel's operating efficiency and can impact upon crew safety. The economic costs are likely to be dependent on the degree of biofouling. If biofouling is significant the pumping efficiency of water on-board a vessel can be reduced. In extreme cases the complete blockage of pipes can compromise the use of vital on-board systems, such as those used for firefighting systems (Palermo 1992). Longer term, biofouling can corrode pipes resulting in unscheduled maintenance (Jones and Little 1990; Grandison *et al.* 2011; Piola and Grandison 2013). The economic impact of biofouling in sea chests and internal pipework is difficult to establish as studies have tended to focus on the more obvious impacts of biofouling on the external hull area (e.g. increased fuel consumption due to increase hydrodynamic drag; Schultz *et al.* 2011).

From a biosecurity perspective, ocean-going vessels have been identified as the major vector for the global translocation of non-indigenous marine species (NIMS) (Bell *et al.* 2011). The biofouling pathway is of particular importance and it has been estimated that 69 – 90 % of established NIMS in New Zealand are likely to have been introduced via this pathway (Cranfield *et al.* 1998). This pattern of introduction is similar for other countries with 70 % of marine species introduced to Hawaii and North America (Eldredge and Carlton 2002; Fofonoff *et al.* 2003), 78 % of introductions in Port Philip Bay (Hewitt *et al.* 2004), and 42 % of unintentional marine introductions to Japan (Otani 2006), are likely to have been

introduced by vessel fouling. The establishment of NIMS can negatively impact upon environmental, social and economic values (e.g. Ruiz *et al.* 1997). Examples of recent introductions to New Zealand that may have occurred via hull fouling include the Asian kelp (*Undaria pinnatifida*), Asian paddle crab (*Charybdis japonica*), Mediterranean fanworm (*Sabella spallanzanii*) and the club tunicate (*Styela clava*) (Bell *et al.* 2011).

Vessel biofouling is not evenly distributed across the surface of a hull, as areas that are protected from a constant laminar flow of water tend to accumulate a higher biomass of organisms (Coutts *et al.* 2003; Coutts *et al.* 2010). The areas of the hull that are not exposed to a constant laminar flow of water, susceptible to antifouling coating system wear or damage, or are infrequently coated are called 'niche' areas. These areas include propellers, rudder shafts, rudder hinges, stabiliser fin apertures, bow and stern thrusters, edge and weld joints, dry docking support strips, cathodic protection anodes, sea chests and internal pipework (Coutts and Taylor 2004; Coutts and Dodgshun 2007; International Maritime Organisation 2011). MAF Biosecurity New Zealand (now the Ministry for Primary Industries) commissioned research on vessel biofouling found that over 80 % of species sampled were found in niche areas (Bell *et al.* 2011). The research concluded that although niche areas account for a relatively small proportion of the submerged hull surface they present a disproportionate biosecurity risk (Coutts and Taylor 2004; Floerl *et al.* 2010; Bell *et al.* 2011; Inglis *et al.* 2013). Once an organism is established in a sea chest its survival may be facilitated by the continuous supply of food (by filter feeding or by feeding on sessile organisms), oxygen and by elevated seawater temperatures related to engine activity which may aid in the persistence of tropical organisms (Coutts and Dodgshun 2007).

2.3. Biosecurity risks of sea chest and internal pipework biofouling

Sea chests and internal pipework have been identified as a hotspot for both sessile and mobile marine organisms (Coutts *et al.* 2003; Coutts and Dodgshun 2007; Lee and Chown 2007; Frey *et al.* 2014). Coutts and Dodgshun (2007) surveyed the sea chests of 42 vessels dry docked in New Zealand and identified 150 different taxa. Of these taxa, 10 % were NIMS that were yet to be established in New Zealand and 35 % were cryptogenic. Similar findings have been observed in Canada; Frey *et al.* (2014) identified 299 taxa in sea chests from domestic and international vessels with approximately 15-20 % being either NIMS or cryptogenic.

Sea chest communities are diverse and may consist of bivalves, polychaetes, hydroids, barnacles, bryozoans, crustaceans, ascidians, gastropods, sea stars, anemones, amphipods, algae and sea grass (Coutts *et al.* 2003; Coutts and Dodgshun 2007; Frey *et al.* 2014). Sea chests provide a distinct habitat that can shelter a number of larger adult organisms that may not be capable of surviving on other areas of the hull or in ballast water (Leach 2011). For example, Coutts and Dodgshun (2007) found five species of live non-indigenous decapods in sea chests of three vessels, with mobile organisms accounting for 42 % of all sampled species. Furthermore, mobile organisms were detected in sea chests of 83 % of vessels sampled (Coutts and Dodgshun 2007), highlighting the range of taxa able to be dispersed via this mechanism and the complexities around suitably treating biofouling in sea chests.

Sediment has also been observed in sea chests (Jones and Little 1990) which may provide habitat for infaunal organisms. Sediment and associated organisms could be entrained when seawater is pumped aboard and may be sourced from the water column, wharf piles and even the seabed in shallow waters (Coutts and Dodgshun 2007).

The translocation of adult marine organisms to new areas increases the likelihood of establishment because they are reproductively mature and can release propagules into the surrounding environment (Godwin 2003). For example, a sea chest surveyed in Australia contained three adult European green crabs (*Carcinus maenas*), of which two were ovigerous females (Coutts *et al.* 2003), and an in-water inspection followed by dry-docking of a Navy vessel in Australia found four adult Asian green mussels (*Perna viridis*) of which one was found attached to a sea chest grate and within that same sea chest 197 juveniles were found (McDonald 2012).

High-risk species to New Zealand that have been found in sea chests of Australian vessels include the European green crab, European clam (*Corbula gibba*) and the Northern Pacific seastar (*Asterias amurensis*) (Coutts *et al.* 2003).

The degree of biofouling in sea chests and internal pipework is independent to that of the external hull and in order to accurately assess a vessel's overall biosecurity risk, these internal areas need to be surveyed. The composition of organisms within vessel sea chests can be relatively unique and is influenced by the geographical regions the vessel has operated within and the ability for organisms to be involuntarily 'vacuumed' into sea-chests when in port (Coutts and Dodgshun 2007). This is unlike the settlement of sessile fouling organisms on vessel hulls. There have been many instances where the general hull can be free of biofouling, yet niche areas contain significant biofouling. This has been highlighted in the Northern Territory of Australia where a 25 m vessel that had an effective antifouling coating was found to be free of external hull fouling, but upon inspection of the internal pipework over 200 individual mussels were found, including the invasive Asian green mussel (*Perna viridis*) and the Asian bag mussel (*Arcuatula senhousia*) (Neil and Stafford 2005). The passenger ferry *Spirit of Australia,* also had a generally clean hull, but large accumulations of macro-fouling were found to be present in the sea chest (Coutts *et al.* 2003). Furthermore, a survey for the NIMS *S. spallanzanii* found none on the general hull but in excess of 100 individuals on the 132 metre container ship *Spirit of Independence* in New Zealand (Katherine Walls pers. comm.). A recent survey of vessels in Canada showed that the biofouling cover and number of taxa significantly increases in a sea chest when the in-service period is > 24 months (Frey *et al.* 2014).

2.4. Preventative approach to sea chest and internal pipework biosecurity

The most effective way to manage biosecurity risk is to prevent the accumulation of biofouling on a vessel (Bax *et al.* 2003; Floerl *et al.* 2005). This is because when NIMS are detected in a new area, they are usually well-established, making containment and eradication costly, labour-intensive and time-consuming (Davidson *et al.* 2008a). For example, the successful eradication of the black striped mussel (*Mytilopsis sallei*) from three marinas in the Australian Northern Territory, cost in excess of AU$ 2.2 million and required the use of a biocide to kill all life in the marinas. In New Zealand incursion responses to *S. spallanzanii* have cost in excess of NZ$ 3.5 million (Ministry for Primary Industries 2015) and eradication has not been successful. In general the eradication of NIMS using current management measures has had limited success (Hewitt *et al.* 2004; Hewitt and Campbell 2007). In 2011 the International Maritime Organization (IMO) published guidelines to minimise the transfer of NIMS via vessel biofouling. For sea chests and internal pipework it was recommended that marine growth protection systems (MGPS) be installed to prevent the settlement of biofouling organisms. Antifouling paints may also be applied to the internal area of a sea chest and the bars of the grates (IMO 2011).

2.4.1. Marine growth protection systems

The most common MGPS utilised in vessel sea chests are sacrificial anodic copper systems (e.g. Cathelco®) and chlorine-based dosing systems (e.g. Chloropac® or Ecocell®) (Grandison *et al.* 2011). Sacrificial anodic copper MGPS normally consist of a pair of copper anodes (for biofouling control) and an aluminium or iron anode (to counteract corrosion of the sea chest and internal pipes). The anodes are placed within the sea chest or strainer box as close to the seawater intakes as possible (Grandison *et al.* 2011). An electrical current is introduced to the anodes which cause the release of copper, aluminium or iron ions into the seawater which are then dispersed throughout the sea chest and internal pipework. The concentration of ions released can be controlled by altering the voltage of the electrical current (Grandison *et al.* 2011). MGPS that are installed in the sea chest will provide protection to both the sea chest and internal pipework, whereas those installed in the strainer will only protect the internal pipework with the sea chest being left unprotected (Chris Scianni pers. comm).

Chlorine-based MGPS use on-board sodium hypochlorite generators. Seawater is pumped through an electrolyser cell and subject to a low voltage electrical current. This causes the chlorine in the seawater to be converted to sodium hypochlorite which is then pumped back into the sea chest for dispersal (Grandison *et al.* 2011). Similar to the copper anode MGPS, the dose of chlorine produced is voltage dependent. Novel MGPS include the use of sonication or ultra-violet light (Grandison *et al.* 2011).

2.4.2. Antifouling paints

Antifouling paints are used to prevent or minimise the settlement of biofouling on submerged surfaces. They are divided into two main categories: biocidal and biocide-free coatings. Biocidal coatings contain chemicals which inhibit larval settlement and attachment. Biocide-free coatings are further divided into fouling-release coatings (which have low surface energy, reducing the strength of biofouling adhesion) and mechanically-resistant coatings (designed to be mechanically cleaned and are resistant to abrasive forces) (Morrisey *et al.* 2013).

Of all the antifouling paints in use, biocidal paints containing copper are the most commonly used following the ban of organotin biocides due to non-target environmental effects (IMO 2005; Grandison *et al.* 2011; Morrisey *et al.* 2013). Biocidal paints work most effectively when they are exposed to a constant laminar flow of water, as this facilitates the continual release of the biocide. Antifouling paints within sea chests, however, are not exposed to a constant laminar flow of water and this influences their ability to prevent the settlement and establishment of biofouling (Morrisey *et al.* 2013). Antifouling coatings using silicone-hydrogel technology that allow for a biocide release-rate independent of vessel speed may present a solution to this problem.

2.5. Efficacy of preventative measures

Despite the use of MGPS and antifouling paints within vessel sea chests and internal pipework, there are multiple published studies that question their efficacy (Coutts and Dodgshun 2007; Grandison *et al.* 2011; Frey *et al.* 2014). For example, Lee and Chown (2007) found the sea chest in the South African vessel *Agulhas* to be severely fouled with the Mediterranean mussel (*Mytilus galloprovincialis*) despite being protected by antifouling paint (containing copper and zinc oxide) and an unnamed cathodic protection system. Lewis *et al.* (1988) tested the efficacy of the Cathelco® sacrificial anodic copper systems on submarine saltwater cooling systems and found that a dose of at least 10 times greater than the

manufacturer's recommendation was needed to control tubeworm biofouling. Issues with increasing the copper dose rate include increases to: power consumption, anode consumption and copper released into the environment. Such issues will increase the cost of using the MGPS. Recently, a vessel survey in Canada showed that the amount of biofouling cover and number of taxa significantly increases within a sea chest when the in-service period is > 24 months (Frey *et al.* 2014), although this survey did not include in its analysis whether a vessel had a MGPS or another preventative biofouling measure installed (e.g. antifouling paint). MGPS can reduce the rate at which biofouling accumulates in sea chests and internal pipework and this protection is dependent on where the MGPS is installed (sea chest or strainer; Chris Scianni pers. comm), however, irrespective of location they tend to be less effective against mobile organisms (Coutts and Dodgshun 2007; Grandison *et al.* 2011).MGPS may not be installed on some vessels due to their excessive cost relative to the biofouling prevention they afford, thus for some vessels the sole preventative biofouling management measure, if any, within sea chests is the application of antifouling paint. Between 2008 and 2011 around 50 - 70 % of vessels arriving in Californian ports reported having MGPS installed (Scianni *et al.* 2013).

Development of strategies to prevent the settling and establishment of biofouling in sea chests and internal pipework is ongoing. For strategies that fail, or for vessels that do not apply them, there exists a need to develop reactive in-water systems that can manage this risk. For example, the Northern Territory (NT) Government of Australia requires that all international vessels that are < 25 m in length have their internal seawater systems dosed with a 5 % detergent solution (Conquest) for a minimum of 14 hours upon arrival in NT(Neil and Stafford 2005). This protocol was established following the 1999 black-striped mussel (*Mytilopsis sallei*) incursion in Darwin, NT and subsequent eradication. Such treatments could also be used as part of a preventative biofouling strategy (i.e. clean/treat before you leave approach).

2.6. Considerations for reactive in-water systems

When considering potential reactive in-water systems for sea chests and internal pipework, a number of factors need to be considered. The internal seawater systems of a vessel are structurally complex, for example the use of internal baffles make it extremely difficult to inspect and clean all internal surfaces (Justin MacDonald pers. comm.). A sea chest on the vessel *Agulhas* had a surface area of approximately 42 m^2 even though the volume of the recess is only 6 m^3. This is due to the complex internal structure which creates a range of potential habitats (Lee and Chown 2007). To accommodate this problem it may be necessary for vessels with complex internal pipework to have multiple access points to ensure removal or treatment system efficacy (Grandison *et al.* 2011).

The following biological factors must be considered when evaluating the efficacy of an in-water system to be applied to sea chests and internal pipework:

- type of biofouling,
- life stage of biofouling,
- organism size,
- density of biofouling, and
- geographic origin of biofouling.

The most successful types of sessile biofouling taxa that have spread to new geographical locations include bivalves (mussels, oysters and clams) and barnacles (Rajagopal and Van der

Velde 2012). This is because they can withstand a wide range of environmental conditions due to their ability to close their shell for an extended period of time and resume feeding once conditions are favourable (Neil and Stafford 2005; Rajagopal 2012). Any system being efficacy tested must include representative taxa from these groups. Further, mobile biofouling taxa (e.g. fish, crabs, sea stars) which are associated with sea chests may detect the treatment (e.g. chemicals, heat) and actively evade it. To date, studies on biofouling control or elimination have not tended to include mobile organisms (Piola and Hopkins 2012).

Heavily biofouled sea chests and internal pipework are likely to contain a number of macro-organisms. These assemblages represent a greater biosecurity risk compared to earlier stages of fouling (e.g. slime layer) because they are likely to contain reproductively mature organisms which are more difficult to remove or render non-viable compared to their larval or juvenile stages (Piola and Hopkins 2012). Further, mature macro-fouling assemblages are three dimensionally complex structures which may make it difficult for a treatment to render each individual organism non-viable.

Organism size can influence treatment efficacy. Piola and Grandison (2013) found that smaller sized Australian blue mussels (*Mytilus galloprovincialis planulatus*) were more resilient and survived in higher numbers when exposed to quaternary ammonium compounds (QACs) compared to larger sized individuals.

The origin of the biofouling will also influence the susceptibility of some organisms to certain treatments (e.g. heat treatment). For example, biofouling species originating from the tropics would be expected to have a greater tolerance to elevated water temperatures compared to species from temperate regions (Piola and Hopkins 2012).

The effect of environmental variables (e.g. temperature, pH, suspended solids) of seawater on treatment efficacy needs to be considered to find out if there are any operational limitations. For example, it is known that that non-oxidising chemicals and chlorine are more effective at higher water temperatures (Rajagopal *et al.* 1995a; Neil and Stafford 2005).

Sea chests and internal pipework which are subject to a treatment that does not physically remove biofouling (such as thermal or chemical treatment) are likely to be re-colonised at an accelerated rate as the calcareous remains of expired organisms provides settlement cues and habitat for larval species (Claudi and Mackie 1993). Oysters cement one of their valves to the substrate after settling as larvae, and it remains attached even after death has occurred (Rajagopal *et al.* 2003a). Leach (2011) found the calcareous remains of several taxa on settling plates that had been subject to thermal treatment. If biofouling is not removed after treatment there is likely to be no operational benefit to the vessel (e.g. increased pumping efficiency). If biofouling is removed there may be some operational advantage, although detached organisms can accumulate as debris and block pipes.

2.7. Environmental risk associated with in-water removal or treatment

The two types of environmental risk associated with removing or treating biofouling in in-water systems are chemical and biosecurity contamination. Both of these risks need to be appropriately assessed and are dependent on a variety of parameters (e.g. level of biofouling and extent of coverage, vessel origin, vessel size, antifouling coating type and age, treatment type (e.g. biocide use); see Morrisey *et al.* 2013). The effluent from an in-water system discharged into the surrounding aquatic environment must meet local and international regulatory requirements with respect to, for example, chemical concentration or temperature

limits (Grandison *et al.* 2011). Due to strict water quality regulations (e.g. California water quality criteria) there has been the incentive for the development of in-water systems with recapture technologies to mitigate both the chemical and biosecurity concerns (Chris Scianni pers comm.; Lewis 2013). Because of these risks, several jurisdictions are developing tool and biosecurity frameworks that will allow in-water cleaning to be appropriately managed whilst also providing guidance for future innovation (e.g. California, Hawaii, Western Australia and New Zealand).

One way to manage the environmental risk when applying in-water systems to sea chests and internal pipework is to isolate the vessel's internal water body by placing a water-tight cover over the sea chest grate. The isolated water could then be pumped on-shore for disposal. Ideally, a sea chest cover would be easy to fit in place, form a robust water-tight seal and be capable of fitting a variety of different sized sea chest grates. Such in-water blanking systems have already successfully been used to isolate the internal areas of a vessel (Justin MacDonald pers. comm.)

2.8. Economic cost associated with in-water systems

The cost and duration of application of an in-water system must also be considered from an economic perspective, as the successful uptake of any system will depend on these factors. In-water systems are likely to be much cheaper than dry-docking a vessel and could result in significant savings if used for regular hull maintenance or as a reactive method to manage biofouling (Floerl *et al.* 2010). Inglis *et al.* (2012) estimated that it will cost NZ$ 5 200, $ 6 500 and $ 7 800 to heat treat sea chests of vessels 50, 100 and 200 m long, respectively. New Zealand is considered to offer relatively cheap dry-docking services compared to other countries with the cost for cleaning at one dry-dock that can take vessels up to 104 m in length ranging from ~NZ$ 9 500–31 000, however, this does not include the cost of antifouling paint application which can cost ~NZ$ 40 000–96 000 depending on vessel size (Floerl *et al.* 2010).

The duration of system application should be the minimum time required to be effective whilst also minimising the disruption to the vessel's schedule (Inglis *et al.* 2012). Neil and Stafford (2005) considered a maximum treatment time of 12 hours (i.e. overnight), as any longer could inhibit the uptake of a particular treatment. Using current methods, the time required to apply an in-water system to the *general hull* area of a vessel 50 metres in length has been estimated to take 1-2 days for mechanical cleaning, 1 day for water blasting, is not specified for heat treatments and is at least 17 hours to 14 days for encapsulation with minimum and maximum times dependant on whether an oxygen scavenging or biocidal chemical is used (Inglis *et al.* 2012; Atalah *et al.* 2016; Morrisey *et al.* 2016).

2.9. Reactive systems for sea chests and internal pipework

The most common way to reactively manage the biosecurity risks associated with sea chests and internal pipework is dry-docking and physical removal and treatment of the fouling. This approach has several limitations including: expense, duration and reliance on the availability of suitable dry-docking facilities (Piola and Grandison 2013). For example, when *Perna viridis* was found on two Australian Navy vessels in 2011 and another vessel in 2012, emergency dry-docking costs were approximately ~AU$ 900 000 per vessel. The minimum time before the vessel was operational again was 7 days (Piola and Grandison 2013). The Australian Navy therefore investigated whether there were alternative in-water options that could be used preventatively or reactively to manage the biosecurity risk while being cheaper

and causing less disruption to vessel itineraries (Piola and Grandison 2013). Also in 2011, the Australian Navy in-water treated the sea chests and internal pipework of a vessel fouled with *P. viridis* using a quaternary ammonium compound. This was achieved by sealing off each sea chest from the external environment and flooding it with the biocide. The apparently successful treatment of all 13 sea chests occurred in < 48 hours, costing approximately ~AU$ 38 000 (Piola and Grandison 2013).

There are a number of reactive in-water systems that have been considered or are in development to manage the biosecurity risks associated with sea chests and internal pipework. These systems, however, all have trade-offs with regard to cost, duration, worker safety, environmental risk and efficacy. There are few systems readily available for use in real-world conditions (Grandison *et al.* 2011; Piola and Grandison 2013).

2.9.1. Learnings and potential for adaptation from land-based systems

Land-based industrial water cooling systems (e.g. power plants, water treatment plants) that use bulk seawater or freshwater are subject to biofouling and the principles applied to its control is continually developing in response to native and introduced species (e.g. the zebra mussel *Dreissena polymorpha*) (Rajagopal and Van der Velde 2012). The economic impact caused by the zebra mussel is estimated to cost the United States of America over US$ 1 billion a year (Costa *et al.* 2012). Most of this cost is related to controlling mussel biofouling to prevent pipe and equipment blockages and due to the reduced efficiency of water cooling systems (Costa *et al.* 2012).

A number of systems developed for use in industrial water cooling systems will likely be capable of being adapted for sea chests and the internal pipework of vessels. For example, systems that are capable of killing or removing biofouling located in enclosed hard-to-reach areas will be ideal candidates for testing on vessels. Before this can occur, the different management goals and operational limitations of the systems need to be considered. For example, industrial water cooling systems typically control biofouling to below a certain operational threshold, and biofouling is dominated by a small number of species (Rajagopal *et al.* 2012). By contrast, a biosecurity treatment for a vessel should require 100 % mortality or removal of a diverse range of soft and hard-bodied species in order to minimise the likelihood of establishment. Also land-based operations do not have space restrictions encountered on vessels and thus can store and use more chemicals or use larger systems (Grandison *et al.* 2011).

There are a variety of in-water systems that are reactively used on biofouling assemblages in industrial water cooling systems, sea chests and internal pipework (Table 1), with chlorine and heat treatments being the most commonly used in industrial water cooling systems (Venkatesan and Murthy 2009).

2.9.2. Applicability of in-water cleaning systems to other scenarios

In-water cleaning systems that are assessed using the developed guidelines and testing framework could be used in other scenarios to treat or clean vessel sea chests and internal pipework. For example, while a vessel is in dry-dock for maintenance the internal water systems could be treated or cleaned, as currently it is difficult to adequately assess all internal spaces. Alternatively, approved systems could be used in-water to manage fouling at an earlier stage of development at regular intervals, preventing the build-up of heavy fouling.

Table 1: Reactive biofouling systems for industrial water cooling systems and vessel sea chests and internal pipework*.

		Industrial water cooling systems		Vessel sea chest / internal pipework	
System category	**Treatment agent / system type**	**Commercially available**	**Experimental**	**Commercially available**	**Experimental**
Chemical: oxidising agents					
	Chlorine	✓		✓	
	Chlorine dioxide	✓			✓
	Ozone	✓			
	Bromine	✓			✓
	Hydrogen peroxide		✓		✓
	Ferrate		✓		✓
	Peracetic acid		✓		✓
	Acetic acid				✓
	Organic tin		✓		
	Descalers (e.g. *Rydlyme*®)			✓	
Chemical: non-oxidising agents					
	Quaternary ammonium compounds	✓		✓	
	Carbamate compounds		✓		
	Cyano-containing compounds		✓		
	Bacteria-based molluscicides		✓		
	Ammonium nitrate		✓		
	Copper ions	✓			
	Potassium salts	✓			
	Sodium metabisulfite	✓			
	Change in pH		✓		
	Carbon dioxide[4]		✓		✓
	Bacterial toxins		✓		
	Natural biocides		✓		
Non-chemical					
	Physical removal	✓		✓	
	Thermal	✓		✓	
	Magnetic field		✓		
	Desiccation	✓			
	Deoxygenation		✓	✓	
	Freshwater (osmotic shock)		✓	✓	

*The table includes treatments that are currently used or are in development (commercially available vs. experimental) and excludes preventative treatments or those that are not yet technologically feasible (Chou *et al.*1999; Grandison *et al.* 2011; Rajagopal *et al.* 2012; Atalah *et al.* 2016). For example, iodine, ozone, acoustics and ultra-violet treatments are not included as they are considered unsuitable for killing or removing established biofouling located in sea chests and the internal pipework of vessels (Grandison *et al.* 2011).

[4] Not a stand-alone treatment but can be used as a co-treatment to enhance effectiveness (Grandison *et al.* 2011).

3. Chemical treatments

3.1. Background

Chemical treatments are commonly employed to preventatively or reactively treat biofouling in vessels and industrial cooling water systems. Chemical treatments are used because they are more versatile and cheaper than most other methods (Costa *et al.* 2012).

The efficacy of a chemical treatment relies on the mode of action, method of delivery, dose and exposure time. Biocidal chemicals are generally classed into two main groups based on their mode of action. Oxidising chemicals attack an organism's cellular integrity, causing cell death and eventually organism mortality, whereas non-oxidising chemicals can cause death in a variety of ways, such as by disrupting cellular transport mechanisms or affecting cellular protein structures (Grandison *et al.* 2011). Oxidising chemicals are able to kill a broad spectrum of biofouling species whereas non-oxidising chemicals are more specific, effective on certain taxa.

The delivery method of chemicals is an important factor as this may affect the final concentration that is distributed throughout the entire sea chest and pipework and also the concentration that is potentially discharged into the environment. It may be difficult to achieve the desired chemical concentration uniformly through structurally complex sea chests and pipework. This could be overcome by having multiple chemical injection points. In addition, the majority ofchemicals are injected into bulk water, where they then diffuse to the interface between the fouled surface and water. Here they accumulate to a concentration high enough to kill the attached organisms. The major drawback of this method is that most of the chemical added is not available at the surface-water interface where it is needed. This results in the application of a higher dose than is actually required to treat the biofouling (Rajagopal 2012).

Some biofouling organisms can detect chemicals that are added to bulk water and will actively respond in order to survive the treatment. One of the most resilient biofouling groups are shelled organisms (e.g. mussels and barnacles), as they can close their shell and stop feeding to prevent the uptake of the chemical and therefore limit soft tissue damage. Upon detecting chlorine in water, mussels are capable of keeping their shells closed and surviving on food stores and anaerobic respiration for up to three weeks (Costa *et al.* 2012). Rajagopal (2012) concluded that shelled organisms are more resilient to chlorine treatment compared to soft-body organisms such as hydroids and ascidians.

One method of biocide delivery developed to counteract the problem of chemical detection is microencapsulation, for example, BioBullets. BioBullets are small (micron-sized) spheres that contain a biocide which is coated in an edible layer that can be ingested by filter feeding biofouling organisms (e.g. *Mytilopsis, Perna, Didemnum, Ciona;* http://biobullets.com/industries/). This delivery method has both environmental and economic benefits as it overcomes the shell closing response of bivalves and thus reduces the required dose, (Costa *et al.* 2012). Such an application method could be used to treat biofouling in sea chests and internal pipework (http://biobullets.com). Independent trials of the BioBullet product SB2000 resulted in 100 % mortality of the brown mussel (*Perna perna*) and the false mussel (*Mytilopsis trautwineana*) following a single dose of 50 mg/L for 48 hours (David Aldridge pers comm.).

The application of watertight covers over the sea chest grates has been successfully used to maintain sufficient concentration of chemicals within the sea chest and internal pipework and to prevent their environmental release. Once in place, the biocide can be pumped into the internal cavity until it is observed to be flowing from the overboard discharges (Piola and Grandison 2013). Compared to a once-through treatment, the application of sea chest covers will help decrease the amount of chemical used and the exposure time required. This also makes meeting any environmental regulations easier as the effluent is contained and can be removed and treated if required.

The exposure time needed to induce biofouling mortality is influenced by the chemical concentration that is administered, with exposure times generally decreasing when higher concentrations are used (Table 2). The dose required to kill established biofouling communities is far higher than that required to prevent settlement (Venkatesan and Murthy 2009).

4. Oxidising chemicals

4.1. Chlorine

4.1.1. Introduction

Chlorine is a non-selective biocide useful for treating biofouling as all organisms are to some degree susceptible (Rajagopal 2012).

Chlorine is the most frequently used biocide to treat aquatic systems. It is a powerful oxidising agent and was first used to disinfect drinking water over 100 years ago (Jenner *et al.* 1998). Additionally, the use of chlorine is likely to play an important role in ballast water treatments (Tsolaki and Diamadopoulos 2009). Because it has been used as a disinfectant for an extended period of time, it is the most extensively studied biocide with regards to its chemistry, toxicity and ecotoxicity (Rajagopal 2012).

Chlorine affects macro-fouling in three ways: by producing toxic effects on adult organisms, by preventing the settlement and growth of larvae, and by reducing the attachment strength of biofouling to the substrate (Mackie and Claudi 2009a).

4.1.2. Chemistry

Chlorination chemistry is very complex due to the large number of reactions (Rajagopal 2012). When added to seawater, chlorine is an active oxidiser, reacting with multiple organic and inorganic substances to produce a large number of chlorinated and brominated compounds which are termed ‘chlorine by-products’. Some of these chlorine by-products are known to persist in the environment and can be toxic, mutagenic or carcinogenic (Khalanski and Jenner 2012). Trihalomethanes are the major by-products of concern, but others include haloacetonitriles, halophenols and haloacetic acids (Allonier *et al.* 1999). Because of these numerous reactions and creation of chlorine by-products, a large portion of the chlorine added to seawater is not available to act as a biocide (Rajagopal 2012).

The term, ‘chlorine residual’ is given to the amount of chlorine and chlorine by-products which still maintain some oxidative power after the numerous side reactions have occurred. The chlorine residual available as a biocide in bulk water consists of two forms: free available chlorine (FAC) (in the form of hypochlorous acid and hypochlorite ion) created by the hydrolysis of chlorine, and chloramines which are formed when chlorine reacts with

ammonia. The most powerful form of FAC against biofouling is hypochlorous acid, as it is uncharged and can diffuse into cells. The chemistry of chlorine is further complicated in seawater because of the presence of bromide. Bromide reacts with free chlorine to form hypobromous acid which then also reacts with ammonia. All of the above oxidants can then react with organic matter to produce toxic and mutagenic halogenated by-products (Kitis 2004; Rajagopal 2012).

4.1.3. Environmental considerations

The non-selectivity of chlorine creates an environmental issue, as discharged water containing residual chlorine can negatively impact upon aquatic ecosystems (Boelman *et al.* 1997). Environmental agencies therefore restrict the concentration of chlorine that is permitted for release into the environment (Rajagopal *et al.* 2012). If discharge limits are stringent, then dechlorination of effluent may be required before discharge can occur (Chou *et al.* 1999). This can be achieved via adding sulphur dioxide or sodium thiosulphate, which reacts with the residual chlorine and neutralises it (Tsolaki and Diamadopoulos 2009; Morrisey *et al.* 2016).

4.1.4. Efficacy of treatment

The efficacy of chlorine is influenced by a variety of biotic and abiotic factors.

Shelled organisms can detect chlorine and close their shell. For example, three mussel species (*Mytilopsis leucophaeata*, *Dreissena polymorpha* and *Mytilus edulis*) reduced valve openings by more than 90 % compared to the control when exposed to 1 mg/L chlorine (Rajagopal *et al.* 2003b). This problem can be overcome by exposing shelled organisms to chlorine for an extended period of time (up to a few weeks). Alternatively, exposure time can be reduced by using higher concentrations of chlorine. Other options include using chlorine in conjunction with a co-treatment that works synergistically to reduce the required exposure time (e.g. carbon dioxide or increased seawater temperature; Rajagopal *et al.* 2002; Venhuis and Rajagopal 2010).

Morrisey *et al.* (2016) used chlorine to treat an 8 metre long yacht. The chlorine, in the granular form of sodium dichloroisosyanurate (dichlor), was applied at an initial concentration of 200 mg/L FAC and the vessel was held within an encapsulated dock for 16 hours. Thirty Mediterranean fanworms (*Sabella spallanzanii*) were collected after the experiment and 28 were judged non-viable after not responding to touch and all had body lesions, and lost or damaged crowns. A further 33 *S. spallanzanii* collected 6 days post-treatment were also non-viable and divers visually observed oyster (*Crassostrea gigas*) shells that were gaping or empty. This outcome occurred despite the concentration of FAC decreasing within the floating dock from 200 mg/L to 50 mg/L after two hours and to < 10 mg/L after 16 hours. Prior to this treatment, the 4 hour EC_{99} for *S. spallanzanii* was calculated to be 160 mg/L FAC.

Rajagopal (2012) found that *Perna viridis* was the most resistant among 10 tropical bivalve species, with 100 % mortality occurring after 816 hours exposure to 1 mg/L chlorine at 29 °C. A concentration of 10 mg/L resulted in 100 % *P. viridis* mortality after 48 hours at the same water temperature (Rajagopal *et al.* 1995a; Rajagopal *et al.* 2003c).

Crassostrea madrasensis, a tropical oyster species, has been found to display a chlorine tolerance below that of *P. viridis* and above that of the brown mussel (*Perna perna*)

(Rajagopal *et al.* 2003a). Rajagopal (2012) found that most shelled organisms succumb to chlorine more rapidly than mussels, so it can be expected that a treatment that induces mortality in mussels will be a good indicator of overall treatment efficacy. It is likely that a chlorine treatment regime that can kill the *P. viridis* will be effective on other bivalve species.

Experience from biofouling control in European water cooling systems has found that when mussels are controlled by chlorination there are no operational problems caused by barnacles (Jenner *et al.* 1998). Although, regardless of the biofouling species present, the efficacy of chlorine treatment may be influenced by fouling biomass (Mackie and Claudi 2009a).

Susceptibility to chlorine varies with organism size; however, the relationship is not consistent between species. An experiment using Conrad's false mussel (*M. leucophaeata*) showed that chlorine tolerance was highest amongst medium-sized individuals while smaller and larger mussels were more susceptible (Rajagopal *et al.* 2002). By contrast, smaller-sized blue mussels (*M. edulis*) are most susceptible to chlorine, with tolerance increasing with size (Rajagopal *et al.* 2005a). The barnacle *Megabalanus tintinnabulum* and oyster *C. madrasensis* display a similar pattern of tolerance (Sasikumar *et al.* 1992; Rajagopal *et al.* 2003a).

Mussels that are attached to surfaces via byssal threads appear more resistant to chlorine compared to those that are not (Rajagopal *et al.* 2005a). Unattached mussels actively attempt to attach by growing byssal threads. During this process they must open their shell to extend their foot thus exposing their soft tissue to chlorine. Conversely, attached individuals can close their shell and recommence aerobic activity once conditions are favourable (i.e. after the chlorine treatment has ceased).

The physiological state of biofouling species influences their susceptibility, with susceptibility increasing during the spawning season for some mussel species (Rajagopal 2012). For example, Conrad's false mussels collected during the spawning season were 29 % more susceptible to chlorine than those collected outside this time. The difference has been attributed to increased filtration rates and hence chlorine uptake of organisms during the spawning season (Jenner *et al.* 1998).

The ability of chlorine to be an efficient biocide is affected by abiotic factors, such as temperature, pH and concentration of suspended solids. The doubling of metabolic activity of ectotherms for every 10 °C increase in temperature facilitates the increased uptake and hence, toxicity of chlorine (Chou *et al.* 1999; Mackie and Claudi 2009a). By contrast, the biocidal effect of chlorine is decreased at pH > 8 as the production of hypochlorite ions are favoured compared to the more effective hypochlorous acid (Rajagopal 2012). Sea water has a pH of 8.1 (Ocean Portal 2015). Further, it is likely that a higher dose of chlorine will be required when operating in near shore environments compared to the open ocean due to the higher concentration of suspended organic and inorganic substances which will reduce the amount of chlorine residual that is available to act as a biocide (Chou *et al.* 1999).

4.1.5. Cost

The 3.6 kilograms of granular dichlor applied in the Morrisey *et al.* (2016) study cost NZ$ 35.

Table 2: Examples of chemical concentration and exposure times required to induce high rates of mortality in adult macro-fouling organisms, with a focus on high-dose treatments.

Treatment or product	Organism type	Species name	Number of replicates	Size (mm)	Dose (mg/L unless stated)	Exposure time (hours unless stated)	Temp (°C)	Mortality (% unless stated)	Reference
Chlorine	Calcareous tubeworm	*Sabella spallanzanii*	1[5]	N/S[6]	200	16	N/S	93[7]	Morrisey *et al.* (2016)
	Mussel	*Perna viridis*	10-15	12 & 95	10	30 & 48	29	100	Rajagopal *et al.* (1995a)
		Perna perna	4	9, 25 & 35	10	82, 102 & 120	29	100	Rajagopal *et al.* (2003d)
		Mytilopsis leucophaeata[8]	~	10	10	168	20	100	Rajagopal *et al.* (1994)
		Brachidontes variabilis	3	7-24[9]	5	27	29	100	Rajagopal *et al.* (2005b)
		Brachidontes striatulus	4	7 & 25	5	102 & 156	29	100	Rajagopal *et al.* (1997)
	Barnacle	*Megabalanus tintinnabulum*	N/S	5 & 30	15	3.3 & 4	28–30	100	Sasikumar *et al.* (1992)
	Oyster	*Crassostrea madrasensis*	4	13, 44 & 65	5	100, 140 & 160	30	100	Rajagopal *et al.*(2003a)
Chlorine dioxide	Mussel	*Dreissena polymorpha*	1	N/S	30 & 40	6.2 & 3.2 minutes	15–20	50	Matissoff *et al.* (1996)
		D. polymorpha	3	N/S	5	70	14.3	100	Holt and Ryan (1997)

[5] Testing occurred on a single fouled yacht
[6] Not stated
[7] 93 % mortality for *S. spallanzanii* sampled directly after treatment. 100 % mortality was observed in *S. spallanzanii* 6 days post-treatment
[8] Original literature was not viewed
[9] Size of mussel had no influence on mortality

Treatment or product	Organism type	Species name	Number of replicates	Size (mm)	Dose (mg/L unless stated)	Exposure time (hours unless stated)	Temp (°C)	Mortality (% unless stated)	Reference
Hydrogen peroxide	Mussel	*D. polymorpha*	1	8-24	40	72	20	100	Petrille and Miller (2000)
		D. polymorpha	3	2–10	30	72 & 576	22 & 12	100	Martin *et al.* (1993)
	Clam	*Corbicula fluminea*	1	9–26	40	216	20	100	Petrille and Miller (2000)
Quatsan®	Mussel	*Mytilus galloprovincialis planulatus*	3	10–92	5 %	24	8.5–31	100[10]	Piola and Grandison (2013)
		M. galloprovincialis planulatus	2	25–65	1, 5 & 10 %	14	16	100[11]	Lewis and Dimas (2007)
Conquest®	Mussel	*Mytilopsis sallei*	3	10–15	1 %	7	29–33	100	Bax *et al.* (2002)
		M. galloprovincialis planulatus	2	25–65	1, 5 & 10 %	14	16	100[12]	Lewis and Dimas (2007)
		M. galloprovincialis planulatus	3	10–92	5 %	24	8.5–31	~90[13]	Piola and Grandison (2013)
Rydlyme®	Mussel	*M. galloprovincialis planulatus*	2	25–65	25 %	24	16	50 % reduction in shell weight	Lewis and Dimas (2007)

[10] 100 % morality occurred 5-7 days after removal from the 24 hour treatment
[11] 100 % mortality occurred 24 hours after removal from the 14 hour treatment
[12] 100 % mortality occurred 48 hours after removal from the 14 hour treatment
[13] ~90 % mortality occurred 5-7 days after removal from the 24 hour treatment

Treatment or product	Organism type	Species name	Number of replicates	Size (mm)	Dose (mg/L unless stated)	Exposure time (hours unless stated)	Temp (°C)	Mortality (% unless stated)	Reference
Hydrochloric (HCl) acid descaler	Mussel	*M. galloprovincialis*	3	40-60	25 %	12	11	100	Bracken *et al.* (in press)
	Mussel	*M. galloprovincialis*	3	40-60	25 %	8	26	100	Bracken *et al.* (in press)
Acetic acid	Mussels and bryozoans	N/S[14]	4	N/S	5 %	48	18-24	100	Atalah *et al.* (2016)
	Calcareous tubeworm	*S. spallanzanii*	1[15]	N/S	N/S[16]	192[17]	19	100	Javier Atalah pers. comm. Cawthron Institute
	Mussel	*M. galloprovincialis planulatus*	6 or 9	N/S	10 %[18]	12	25	75	Neil and Stafford (2005)
		M. galloprovincialis planulatus	2	25-65	10 & 50 %[19]	6	16	100[20]	Lewis and Dimas (2007

[14] Not stated
[15] Testing occurred on a fouled vessel
[16] During the course of the treatment 220 litres of glacial acetic acid was used
[17] Prolonged exposure time was due to the inability to isolate encapsulated water from the surrounding environment
[18] Vinegar concentration containing 6 % acetic acid
[19] Vinegar concentration containing 4 % acetic acid
[20] 100 % mortality occurred 48 hours after removal from the 6 hour treatment

4.2. Chlorine dioxide

4.2.1. Introduction

Chlorine dioxide is considered a more powerful oxidant than chlorine (Rajagopal *et al.* 2012), and it has been used as a water disinfectant for more than 50 years (Mackie and Claudi 2009a). Chlorine dioxide exists in the form of a gas and for water treatment applications chlorine dioxide tablets can be used although when high concentrations are required on-site generation is recommended due to its hazardous nature (Mackie and Claudi 2009b). Chlorine dioxide can be generated using specialised equipment from a variety of precursors such as: sodium chlorite and hydrochlorite acid; sodium chlorite and chlorine gas; and, sodium chlorite and sodium hypochlorite (Mackie and Claudi 2009b).

4.2.2. Chemistry

Chlorine dioxide in solution does not react with bromine or ammonia, meaning there are fewer side-reactions compared to chlorine. Chlorine dioxide does oxidise with metals in reduced forms (Fe^{2+}, Mn^{2+}), nitrites (NO_2-) and sulphites (SO_2-) and dissolved organic matter (Dore 1989). In polluted or eutrophic waters these oxidising reactions can reduce the amount of chlorine dioxide that is available for use as a biocide (Rajagopal *et al.* 2012). This 'demand' must be considered because reactive in-water treatments are most likely to take place in coastal areas (ports) where seawater is more likely to contain organic substances.

4.2.3. Environmental consideration

The by-products generated by oxidising reactions of chlorine dioxide in solution mainly consist of sodium chlorite, chlorate and chloride, which are generally considered acceptable for discharge by regulatory bodies (Mackie and Claudi 2009b).

4.2.4. Efficacy of treatment

There are very few data on the efficacy of chlorine dioxide for use as a reactive measure for macro-fouling. Most studies on industrial water cooling systems have focused on preventative treatment via continuous application (Mackie and Claudi 2009b).

4.2.5. Cost

As a biofouling treatment, chlorine dioxide costs approximately 2.5 times more to use compared to chlorine (Venkatesan and Murthy 2009; Grandison *et al.* 2011).

4.3. Bromine

4.3.1. Introduction

Bromine has primarily been used as a water treatment method for swimming pools (Chou *et al.* 1999).

4.3.2. Chemistry

As a biocide, bromine can be used in several different forms including activated bromine, sodium bromide, bromine chloride and proprietary solutions of bromine with other chemicals (e.g. chlorine) (Mackie and Claudi 2009b). The biocidal property of bromine is similar to that

of chlorine in both action and effectiveness, with the oxidising ability of bromine increasing when the pH > 8 (Mackie and Claudi 2009b).

4.3.3. Environmental considerations

Several toxic by-products are formed when bromine is added to seawater, although these may rapidly degrade, potentially limiting the environmental impact (Grandison *et al.* 2011).

4.3.4. Efficacy of treatment

Bromine is often added to chlorine treatments to enhance its biocidal effect, especially in mildly alkaline waters (Sprecher and Getsinger 2000; Grandison *et al.* 2011). There is an absence of studies assessing the efficacy of bromine as a sole macro-fouling treatment, although Mackie and Claudi (2009b) state that as a rough guide, the amount of oxidant required is the same as for chlorine.

4.3.5. Cost

The cost of bromine treatment may limit its use as it is approximately twice the cost of chlorine and offers a similar level of efficacy (Chou *et al.* 1999; Venkatesan and Murthy 2009).

4.4. Hydrogen peroxide

4.4.1. Introduction

Hydrogen peroxide is used principally as a biocide in small contained systems, such as fuel bays in nuclear power stations (Mackie and Claudi 2009).

4.4.2. Chemistry

Due to its rapid degradation in seawater and inactivation by bacterial enzymes, in order to effective hydrogen peroxide needs to be applied at relatively high doses and at low temperatures (Jenner *et al.* 1998; Grandison *et al.* 2011). As a result, it is difficult to treat large bodies of water (Rajagopal *et al.* 2012).

4.4.3. Environmental considerations

The ability of hydrogen peroxide to degrade rapidly to environmentally benign oxygen and water is an advantage from an environmental release perspective (Grandison *et al.* 2011). Further, hydrogen peroxide can be stored in a liquid form, making handling, storage and application simple and safe.

4.4.4. Efficacy of treatment

There is a paucity of literature available on the efficacy of hydrogen peroxide for treatment of established biofouling. Petrille and Miller (2000) reported a 90 % mortality rate of adult zebra mussels (*Dreissena polymorpha*) after exposure to 5.4 mg/L for 21 days. At higher concentrations of 10, 20, and 40 mg/L, 100 % mortality was achieved after 7.8, 8.8 and 3 days, respectively. The Asian clam (*Corbicula fluminea*) appears to be more resilient to hydrogen peroxide at the same tested concentrations, with 100 % mortality achieved after 13.5, 9.5 and 9 days, respectively.

4.4.5. Cost

Compared to chlorine, hydrogen peroxide is less effective, which increases application costs (Chou *et al.* 1999). For this reason alone hydrogen peroxide would not be recommended as an in-water treatment (Claudi and Mackie 1993).

4.5. Ferrate

4.5.1. Introduction

In the 1970s ferrate was investigated as a biocide that could potentially replace chlorine, but it was deemed not economically viable due to the cost of manufacture (Mackie and Claudi 2009b).

4.5.2. Chemistry

Ferrate is considered a more powerful oxidant than chorine, ozone and bromine, as it has a higher redox potential (Mackie and Claudi 2009b).

4.5.3. Environmental considerations

Ferrate in the form of potassium ferrate is considered the safest for use as a biocide due to its ease of production, stability and lack of harmful by-products (Sharma 2002).

4.5.4. Efficacy of treatment

On-site production of ferrate is now possible through the patented Ferrator® system which produces ferrate in a liquid form that can be added to water. This system requires the addition of precursor chemicals (sodium hydroxide, sodium hypochlorite and ferric chloride) which are hazardous (Grandison *et al.* 2011). Recent trials investigating the effectiveness of the Ferrator® system have concentrated on ballast water treatment. Its use as a reactive biofouling treatment has been considered, although there is a paucity of data supporting its efficacy (Mackie and Claudi 2009b).

4.5.5. Cost

Using this new production system has been claimed to reduce costs by more than 90 % compared to previous manufacturing methods (Ferrate Treatment Technologies 2014).

4.6. Peracetic acid

4.6.1. Introduction

Peracetic acid is used as a disinfectant to eliminate harmful micro-organisms in waste water systems (Cristiani 2005).

4.6.2. Chemistry

The mode of action of peracetic acid involves the production of oxygen free radicals that break chemical bonds in cell membrane enzymes (Jenner *et al.* 1998). Peracetic acid is corrosive and unstable at high concentrations and after decomposition its by-products are methane, carbon dioxide, oxygen and water (Rajagopal *et al.* 2012). Peracetic acid does not

exist as a pure compound, and when in aqueous solution it is an equilibrium mixture of acetic acid and hydrogen peroxide.

4.6.3. Environmental considerations

When peracetic acid is applied to seawater it does not persist and does not produce mutagenic by-products when reacting with organic material, making it appealing from an environmental perspective (Kitis 2004; Cristiani 2005). It is a worker safety risk, however, due to it being unstable and corrosive (Grandison *et al.* 2011).

4.6.4. Efficacy of treatment

Peracetic acid appears to be less effective than chlorine at controlling biofouling. The recommended dosage in industrial cooling water systems is in the range of 1–10 mg/L with a 1–3 hour contact time (Jenner *et al.* 1998).

4.6.5. Cost

Peracetic acid is approximately 10-20 % more expensive than compared to chlorine (Venkatesan and Murthy 2009; Grandison *et al.* 2011).

4.7. Acetic acid[21]

4.7.1. Introduction

Acetic acid is the active ingredient within vinegar and has traditionally been used as a household disinfectant. Recently, it has been considered for use as a biocide for the treatment of biofouling (Carver *et al.* 2003; Forrest *et al.* 2007; Denny 2008; Piola *et al.* 2010; Rolheiser *et al.* 2012; Atalah *et al.* 2016).

4.7.2. Chemistry

Acetic acid is a weak acid and its biocidal activity is not alone related to the number of free hydrogen ions present in solution but also on the anions and undissociated molecules which may act independently of pH (Reid 1932).

There is supported by recent evidence that the biocidal effects of acetic acid is a function of the compound itself rather than that of altered pH (Forrest *et al.* 2007; Cortesia *et al.* 2014)

4.7.3. Environmental considerations

Acetic acid is rapidly biodegradable in water (Kitis 2004). Although the use of glacial acetic acid (99 % concentration) which is used when a large volume of water is to be treated has transport and handling risks associated with it being an irritant and because it is mildly corrosive to metals (Morrisey 2015). Acetic acid at a concentration of 5 % (typical of household vinegar) is considered non-toxic and non-irritating (Dvorak no date).

4.7.4. Efficacy of treatment

The use of acetic acid as a biofouling biocide has been experimentally tested on biofouling at small scales (e.g. 20 cm x 20 cm experimental settlement plates; Piola *et al.* 2010; Atalah *et al.* 2016). For encapsulation treatment of multi-species biofouling, Atalah *et al.* (2016) recommend that a concentration of 5 % acetic acid be applied for 48 hours.

[21] *Erratum 2019: The authors of this paper acknowledge that acetic acid is a non-oxidising acid.*

Acetic acid was used to treat *Sabella spallanzanii* on the launch *Columbus*. A large volume (220 litres) of glacial acetic acid (99 %) was applied to the encapsulated water over 8 days. Biofouling was not killed initially due to the encapsulated water not being effectively isolated from the surrounding environment. Subsequent repairs to the PVC wrapping used for encapsulation on the 5th day and addition of 100 litres of additional glacial acetic acid created anoxic conditions which were maintained for a further 3 days and caused total mortality of biofouling as determined by in-water diver visual surveys (Javier Atalah pers. comm. Cawthron Institute). Dissolved oxygen and pH were measured at regular intervals throughout the treatment instead of acetic acid concentration.

4.7.5. Cost

When treating biofouling at the scale of a vessel the use of acetic acid (in the form of glacial acetic acid) is not recommended due to the availability of cheaper and safer biocides (e.g. chlorine in the form of sodium dichloroisocyanurate), although sodium diacetate has been identified as having fewer logistical and safety concerns (Morrisey 2015).

4.8. Descalers[22]

4.8.1. Introduction and chemistry

Descalers are used to remove accumulated insoluble deposits from the internal surfaces of pipes. The active chemical substance is usually an acidic compound, such as hydrochloric or phosphoric acid, which reacts with carbonate compounds producing carbon dioxide and soluble salts (Lewis and Dimas 2007). Descalers can degrade the calcium carbonate shells of fouling organisms.

4.8.2. Environmental considerations

The environmental concerns associated with the handling and discharge of descalers will depend on the active ingredient of the proprietary product. For example, the descaler *Rydlyme*® is advertised as being non-toxic and non-hazardous and can be disposed of in wastewater systems (http://www.apexengineeringproducts.com/products/*Rydlyme*®descaler/). Although, its use in-water on vessels in Western Australia is dependent on it being contained and disposed of on land (Justin MacDonald pers. comm.)

4.8.3. Efficacy of treatment and cost

Neil and Stafford (2005) assessed the ability of *Rydlyme*® to kill the oyster *Saccostrea glomerata* but found it was ineffective.

Lewis and Dimas (2007) tested the ability of three proprietary descalers to kill the Australian blue mussel (*Mytilus galloprovincialis planulatus*). The descaler *Rydlyme*® was the most effective at dissolving shells, with a 50 % reduction in the initial weight of mussels occurring at a 25 % concentration. The effectiveness of descalers is dependent on the availability of acid in solution. A 25 % solution of *Rydlyme*® was required to digest one mussel and to digest others would require a linear increase in acid proportional to the increase in the biomass of the mussels. For example, to digest two mussels would require a 50 % solution of *Rydlyme*®.

[22] *Erratum 2019: The authors of this paper acknowledge that some acids used as active ingredients of descalers are in fact non-oxidising acids.*

Bracken *et al.* (in press) assessed the ability of seven commercially available descalers to dissolve the calcium carbonate shells of *Mytilus galloprovincialis* within a laboratory setting. At 11 °C they found that hydrochloric acid (HCl) based descalers performed better than those containing phosphoric acid or acid-surfactants. Interestingly, increasing the concentration of the descaler above 25 % had a negligible impact on the rate of shell dissolution, with the majority of dissolution occurring within the first 12 hours of exposure. Follow-up experiments achieved 100 % mortality after mussels were exposed to an HCl descaler in a static system for 12 hours (concentration 25 %, 11 °C).

Lewis and Dimas (2007) concluded that for heavily fouled surfaces the volume of descaler required is impractical from both an efficacy and economic standpoint. The findings of Bracken *et al.* (in press) may offer a way forward.

4.9. Category summary

4.9.1. Biosecurity risks

The following biosecurity risks have been identified as associated with in-water treatment of sea chests and internal pipework using oxidising chemicals:

- material may be dislodged from the sea chest grates by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system itself or the equipment used to blank off the sea chests,
- containment associated with the initial application of treatment to achieve the target concentration,
- containment associated with equipment used to blank off the sea chests, and
- efficacy of the system treating the biofouling.

4.9.2. Feasibility

The use of oxidising chemicals for the treatment of pipework is widespread within land-based industrial processes. Such chemicals are also applied to prevent the settlement of biofouling in sea chests and internal pipework. It is therefore appropriate to develop performance standards and testing requirements for in-water systems using oxidising chemicals to reactively treat biofouling in sea chests and internal pipework.

5. Non-oxidising chemicals

5.1. Background

Non-oxidising chemicals generally work by interrupting metabolic processes within an organism (Grandison *et al.* 2011). For many of these chemicals the mode of action is not completely understood (Chou *et al.* 1999; Neil and Stafford 2005).

A single non-oxidising chemical is likely to be only effective at treating discrete groups of organisms (e.g. molluscs). Therefore to control diverse biofouling assemblages the use of a number of non-oxidising chemicals or different systems may be required (Grandison *et al.* 2011).

One advantage of using non-oxidising chemicals is that bivalves will continue to filter feed until detrimental effects occur (Rajagopal *et al.* 2012). Further, these chemicals do not seem to corrode piping (Grandison *et al.* 2011).

Non-oxidising chemicals, in addition to being taxon-specific, are expensive and likely to be less effective than chlorine (Chou *et al.* 1999; Grandison *et al.* 2011).

In industrial water cooling systems, this class of chemicals is predominately used in closed water systems and can be effective after relatively short durations (12–48 hours) (Rajagopal *et al.* 2012).

5.2. Quaternary ammonium compounds

5.2.1. Introduction

Quaternary ammonium compounds (QACs) are selective biocides that have been traditionally used as anti-bacterial disinfectants and as a biofouling treatment in industrial cooling water systems (Piola and Grandison 2013). QACs have been used to reactively treat biofouling of sea chests and internal pipework on international yachts arriving in Northern Territory (Australia) (Neil and Stafford 2005) and on Royal Australian Navy vessels (Piola and Grandison 2013).

5.2.2. Chemistry

QACs are the most commonly used non-oxidising chemical for treating biofouling (Grandison *et al.* 2011). Biocidal activity of QACs is related to their interruption of metabolism by attaching to negatively charged surfaces such as cell walls and membranes. The exact mode of action, however, requires further research (Neil and Stafford 2005).

5.2.3. Environmental considerations

Two commonly used proprietary formulations for marine applications are Conquest and *Quatsan*®. These are commercial-grade disinfectants containing surfactants, alkaline salts and the QAC benzalkonium chloride. Benzalkonium chloride is rated to be of moderate toxicity (Jenner *et al.* 1998; Chou *et al.* 1999). In general, QACs need to be applied in high volumes to achieve the desired efficacy (Grandison *et al.* 2011).

The use of QACs as an in-water treatment has raised concerns about their ability to persist in the environment and adversely affect aquatic organisms. QACs are not metabolised by aquatic organisms but may accumulate in the edible tissues of fish (Jenner *et al.* 1998). Immobilisation of QACs in soil prevents the contamination of ground (Jenner *et al.* 1998). QACs are capable of being absorbed on suspended matter in water or on colloids such as humic acids. This behaviour means that the concentration of active compounds in solution can be reduced or detoxified by adding clay at a concentration of 5–40 mg/L (Jenner *et al.* 1998). Neil and Stafford (2005) estimated that to achieve safe release into the environment 8 000 L of seawater would have to be added to dilute 1 L of 5 % *Quatsan*® effluent.

QACs appear to cause minimal damage to infrastructure (Piola and Grandison 2013).

5.2.4. Efficacy of treatment

The effectiveness of QACs is influenced by water temperature, with higher temperatures enhancing physiological activity and biocide uptake (Jenner *et al.* 1998).

Following the incursion and subsequent eradication of the black-striped mussel (*Mytilopsis sallei*) in Darwin in 1999, all vessels < 25 m in length were required to have their internal pipework treated with a 5 % solution of Conquest for a minimum of 14 hours (Neil and Stafford 2005). Conquest was used as the treatment option of choice, as a 1 % concentration was found to induce 100 % mortality in black-striped mussels after 7 hours (Bax *et al.* 2002). By contrast, Neil and Stafford (2005) found that *Quatsan*® was only capable of killing ~10 and ~20 % of the Sydney rock oyster (*Saccostrea glomerata*) after a 12 hour exposure to 5 and 10 % solutions, respectively.

Lewis and Dimas (2007) tested the biocidal efficacy of Conquest and *Quatsan*® on the Australian blue mussel (*Mytilus galloprovincialis planulatus*). For all concentrations of Conquest tested (1, 5 and 10 %), 100 % mortality occurred within 48 hours after a 14 hour exposure to the test solution. For *Quatsan*® treatments, all mussels died within 24 hours of the 14 hour exposure to the test solution. The authors also observed that the large amount of foam produced during testing may have had an effect on toxicity.

Piola and Grandison (2013) tested the ability of Conquest and *Quatsan*® to kill the Australian blue mussel in a replica 35 L sea chest and attached piping system. No treatment concentration (1, 2 and 5 %) was effective at causing 100 % mortality after the 24 hour exposure period. Small mussels (0–30 mm) were found to be more resistant and 100 % mortality was only achieved 5 days after a 24 hour exposure to a 5 % *Quatsan*® solution. In large mussels (50–90 mm) 100 % mortality was achieved 5 days after exposure to all concentrations, except the 1 % Conquest. Small mussels were observed to be more sensitive to the presence of the biocide and shut their shell for the duration of the exposure. Following this study it was recommended that the Royal Australian Navy *Quatsan*® dosing protocol for controlling mussels is changed to a 5 % treatment for 24 hours compared to the current dosing guideline of 1 % for 14 hours. As this regime would occur under variable field conditions, it is not expected to result in 100 % mortality of fouling mussel species but would result in higher mortality rates compared to using a 1 % dose.

5.2.5. Cost

The use of QACs to treat biofouling may be more expensive than other treatment options due to the high doses required, the high cost of the proprietary formulations (Grandison *et al.* 2011) and the organism specificity associated with its toxicity.

5.3. Category summary

5.3.1. Biosecurity risks

The following biosecurity risks have been identified as associated with in-water treatment of sea chests and internal pipework using non-oxidising chemicals:

- material may be dislodged from the sea chest grates by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system itself or equipment used to blank off the sea chests,

- containment associated with the initial application of treatment to achieve the target concentration,
- containment associated with equipment used to blank off the sea chests, and
- efficacy of the system treating the biofouling.

5.3.2. Feasibility

Non-oxidising chemicals have previously been applied to reactively treat biofouling in sea chests and internal pipework. It is therefore appropriate to develop performance standards and testing requirements for in-water systems using non-oxidising chemicals to reactively treat biofouling in sea chests and internal pipework.

6. Non-chemical systems

6.1. Physical removal

6.1.1. Introduction

Physical removal of biofouling encompasses a variety of methods, which can include handheld tools (powered and non-powered), brushes, cutting heads, water jets, diver operated carts, remote operated vehicles (ROVs) and robots (Morrisey *et al.* 2014). Rotating brush systems are the most common mechanical cleaning system for hull surfaces (Inglis *et al.* 2012).

Physical removal systems are best suited for treating biofouling on flat or slightly curved external hull surfaces and are likely to be limited in their ability to clean sea chests and internal pipework. The main reason is due to the limited accessibility of all fouled surfaces, particularly within the internal pipework (Inglis *et al.* 2012).

In-water physical removal methods may be able to treat some surfaces within the sea chest if the grate could be removed, although diver safety may preclude this from occurring and the complex internal structures may limit the surfaces that can be accessed. If the sea chest is not completely isolated from the external environment, some mobile organisms may escape treatment.

6.1.2. Environmental considerations

The major drawback with physical removal methods is that the biofouling and mobile organisms needs to be contained to prevent dispersal and establishment in the receiving marine environment (Woods *et al.* 2007). Further there may be chemical contamination of the environment associated with the cleaning of antifouling paints (Morrisey *et al.* 2013).

6.1.3. Efficacy of removal

Physical removal methods have recently been reviewed by Morrisey and Woods (2015). Hull-based physical removal systems are likely to only be effective and operationally practical when treating certain areas of the sea chest (e.g. sea chest grates) (Inglis *et al.* 2012).

An example of a physical removal method that may be suitable for this use is the ‘magic box’ treatment system. Lewis (2013) reported a successful preliminary trial of this system which consists of a transparent removable plastic box that can fully isolate the area of the hull it is

covering. After the hull surface is isolated a high pressure 5 000 PSI water lance or hand scraper tool can be inserted through access ports in the box. Once the biofouling has been removed from the hull it is filtered through a two-stage system and ultra-violet (UV) sterilised. Trials have achieved filtration to 12.5 μm (Morrisey and Woods 2015).

It is unlikely that there is a physical method capable of treating the entire sea chest and internal pipework due to limited accessibility and the complex structure of such areas. Therefore, it is likely that any physical removal method will need to be used in conjunction with another treatment type that can adequately treat fouling on isolated internal surfaces.

6.1.4. Cost

Inglis *et al.* (2012) has estimated that it would cost ~ NZ$ 4 000 per day to clean the external surface of a vessel sea chest grate using a prototype brush system, plus an additional mobilisation cost of ~ NZ$ 2 000.

6.1.5. Category summary

6.1.5.1. Biosecurity risk

The following biosecurity risks have been identified as associated with the in-water removal of biofouling from sea chests and internal pipework:

- material may be dislodged from the sea chest by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system or equipment used to blank off the sea chests,
- efficacy of the system removing the biofouling,
- capture of waste material removed (where a capture system is fitted),
- filtration of captured waste (where a filtration system is fitted) and how effectively, and to what minimum particle size, material is removed from the effluent stream, and
- treatment of effluent (for example, with heat, ultra-violet light or biocides) or discharged to a sewerage system with secondary treatment.

6.1.5.2. Feasibility

In-water systems have previously been applied as a reactive measure to remove biofouling from vessel hulls, however, sea chests and internal pipework present operational difficulties not easily overcome. While these difficulties are acknowledged, the key advantage of these systems is that the biofouling is actually removed allowing sea chests and internal pipework to function effectively. It is, therefore, appropriate to develop performance standards and testing requirements for in-water systems that remove biofouling in sea chests and internal pipework.

Table 3: Advantages and limitations of reactive systems to remove or treat biofouling in sea chests and internal pipework (Bracken *et al.* in press; Forrest *et al.* 2007; Venkatesan and Murthy 2009; Grandison *et al.* 2011; Morrisey 2015).

System type	Advantages	Limitations
Chlorine	Proven biocide with well-established technology. Relatively inexpensive. Can be generated directly from seawater. Wide spectrum of activity. Rapidly loses toxicity without bioaccumulating	Efficacy affected by pH, temperature and suspended solids. Can be corrosive to CuNi pipes. Potential environmental risks associated with discharge. Chlorine discharge is regulated. Forms toxic by-products. Bivalves can detect chlorine and cease aerobic activity which prolongs survival. Biofouling may remain attached to the fouled surface. Worker safety concerns.
Bromine	More effective than chlorine at higher pH. Wide spectrum of activity. Can be used in conjunction with other treatments (e.g. chlorine) to increase efficacy.	Requires a high concentration. Can be consumed quickly. Forms toxic by-products. Approximately twice the cost of chlorine. Potential environmental risks associated with discharge. Biofouling may remain attached to the fouled surface. Worker safety concerns.
Chlorine dioxide	Stronger biocide than chlorine. Technology is well-established. Low corrosion rate of pipes. Efficacy not as influenced by water chemistry as chlorine. Potential for use as a co-treatment. Minimal environmental impact.	Approximately twice the cost of chlorine. Cannot be generated from seawater. Biofouling may remain attached to the fouled surface. Worker safety concerns.
Hydrogen peroxide	Highly reactive. Rapid degradation (minimal environmental concern).	High concentrations needed due to rapid degradation.

System type	Advantages	Limitations
	Readily available.	May form heat and vapour. Less effective than chlorine. Biofouling may remain attached to the fouled surface.
Ferrate	Stronger oxidant than ozone, chlorine and bromine. No by-products. Easy to produce. Stable.	Untested technology for macro-fouling control. Efficacy unknown. Requires handling of hazardous precursor chemicals. Worker safety concerns. Biofouling may remain attached to the fouled surface.
Peracetic acid	Only low concentrations required. Wide-spectrum biocide.	Corrosive. Unstable. Requires handling of hazardous chemicals. Worker safety concerns. Less effective than chlorine. Costs more than chlorine. Biofouling may remain attached to the fouled surface. Potential environmental risks associated with discharge.
Acetic acid	Stable in the presence of organic matter. Easy to produce.	Glacial acetic acid costs more than chlorine. Glacial acetic acid requires handling of hazardous chemicals. Biofouling may remain attached to the fouled surface. Worker safety concerns when using high concentrations.
Descalers	Removes calcareous biofouling through the dissolution of calcium carbonate shells.	Large doses required. Efficacy influenced by biofouling biomass. Requires handling of potentially hazardous chemicals.

System type	Advantages	Limitations
		Expensive compared to chlorine. Potential environmental risks associated with discharge. Can be corrosive to pipework.
Non-oxidising biocides	Non-corrosive. Effective against target organisms.	High specificity of biocides. Multiple biocides often required to kill diverse biofouling assemblages. Long contact times required. Large doses required. Expensive compared to chlorine. Organism resistance may develop. Can be less effective than chlorine. Biofouling may remain attached to the fouled surface. Potential environmental risks associated with discharge.
Physical removal	Removal of fouling provides operational advantage to vessel. Reduces the rate of re-settlement.	Limited to removing biofouling from exposed hull areas. Difficult to access niche areas and internal pipework. Difficult to contain chemical effluent and dislodged biofouling. Recapture of removed biofouling and environmental release of biocides from cleaned surfaces.
Thermal	Reduced environmental impact compared to some chemicals. Broad-spectrum treatment capable of killing all biofouling.	Large energy requirement if heating water. Uniform exposure difficult to achieve. Can facilitate the formation of carbonate scale. Thermal tolerance can occur if sub-lethal exposure occurs.

System type	Advantages	Limitations
		Biofouling may remain attached to the fouled surface.
Deoxygenation	Environmentally benign when applied without the use of chemicals. Established principle. Conceivably can treat any sized vessel.	Long exposure time required (days to weeks). Could promote anaerobic growth of micro-organisms. Can require an oxygen scavenging chemical to accelerate treatment. Requires the isolation of the water body. Deoxygenation promotes the growth of corrosion-inducing bacteria. Biofouling may remain attached to the fouled surface.
Freshwater (osmotic shock)	Environmentally benign.	Requires long exposure time. Large volume of freshwater needed. Biofouling may remain attached to the fouled surface. Could induce spawning of some organisms
Co-treatments	Reduced exposure times. Improved efficacy. Lower temperature and biocide load in bulk water compared with single treatments.	May require additional equipment. Increased cost. Potential environmental risks associated with discharge. Biofouling may remain attached to the fouled surface.

6.2. Thermal treatment

6.2.1. Introduction

The use of heat as a biofouling treatment has been used extensively in industrial water cooling systems (McMahon *et al.* 1995; Boelman *et al.* 1997; Rajagopal *et al.* 2012).

Grandison *et al.* (2011) recommend that thermal treatment be investigated as a reactive shore-based biosecurity treatment for fouled vessels. This treatment also has the potential to be used as a preventative measure, although repeated applications may have unintended consequences to the efficacy of antifouling paints.

6.2.2. Environmental considerations

Thermal treatment is generally considered more environmentally sustainable compared to the addition of biocides, although there may be restrictions on the volume of heated effluent that can be discharged into the surrounding marine environment (Perepelizin and Boltovskoy 2011).

6.2.3. Efficacy of treatment

It is important when testing the efficacy of a thermal treatment that the most tolerant species are assessed. Bivalve species are considered to be more thermally tolerant than other biofouling species, thus thermal treatments that are effective against bivalves should also be effective against most other species (Rajagopal and Van der Velde 2012). For example, a comparison of the thermal tolerances of three frequent fouling species in the Netherlands (*Mytilus edulis, Mytilopsis leucophaeata, Crassostrea gigas*) found that the exposure time needed to cause 100 % mortality was highest for *C. gigas* (Rajagopal and Van der Velde 2012). *C. gigas* was also the most tolerant species in the Piola and Hopkins (2012) study where 100 % mortality of adults were achieved at 57.5 °C for 60 minutes or 60 °C for 30 minutes. By contrast Rajagopal *et al.* (2005c) found that 100 % mortality occurred following exposure to 42 °C after 62 minutes. Of the bivalve species tested, the oyster *Crassostrea madrasensis* is the most thermally tolerant. Rajagopal and Van der Velde (2012) found that 100 % mortality of all bivalve species can be achieved by raising the temperature to 42 °C for approximately (2 hours).

The effect of heat treatment on marine bivalve species (and many other organisms) generally follows a pattern consistent with a steep increase in mortality within a narrow temperature range (Rajagopal and Van der Velde 2012). In bivalves, the response to elevated temperature has been either all organisms survive or are killed. There appears to be very little variation from one individual to the other when sourced from the same population and any individual variation tends to decline as the treatment temperature increases (Graham *et al.* 1975; Rajagopal and Van der Velde 2012).

Other than taxa related differences, the time and temperature requirement for mortality of biofouling organisms is mainly dependent on the acclimation temperature (i.e. the difference between the ambient and treatment temperature) (Table 4; Venkatesan and Murthy 2009).

Mussels can readily acclimatise to changes in water temperature, which influences their acute and chronic lethal temperature limits (e.g. Boelman *et al.* 1997). This means that in summer months a higher maximum temperature will be needed to achieve mortality compared to

winter months when water temperatures are lower. For example, zebra mussels (*Dreissena polymorpha*) acclimatised to 30 °C and heated at 1 °C per minute suffered 100 % mortality at 43 °C. By contrast, when acclimatised to below 20 °C and heated at the same rate 100 % mortality occurred at 38 °C (McMahon and Ussery 1995).

Mussel size can influence thermal tolerance, with smaller zebra mussels being more resilient (Boelman *et al.* 1997), although this size effect is not consistent among bivalve species, e.g. smaller individuals of *C. madrasensis* are less resilient compared to larger ones (Rajagopal *et al.* 2012).

Both acute and chronic strategies are employed for the thermal treatment of biofouling in industrial water cooling systems. The choice strategy depends on a number of factors which would also be relevant to treating vessels.

Acute thermal treatment is defined as the temperature at which death occurs when the water temperature is increased at a specific rate. This involves heating water to a lethal temperature followed by a rapid return to the pre-treatment ambient water temperature. This strategy can be employed in systems where it is difficult to maintain a required temperature for an extended period of time. The advantage of this strategy is that achievement of the required temperature only needs to be confirmed rather than requiring the long-term precise temperature regulation and measurement (Boelman *et al.* 1997).

The temperature within a vessels' sea chest and internal pipework can be monitored through the use of a thermistor (placed by a diver within the sea chest cavity) or a hand-held laser thermometer. The latter can measure the temperature of the external steel surfaces of the sea chest and internal piping. Recording the temperature on external steel surfaces provides good evidence that an even distribution of heat has been applied to the internal surfaces (Rob Hilliard pers. comm.).

The second thermal treatment strategy is to expose biofouling to a chronic upper thermal limit for an extended period of time (Boelman *et al.* 1997). The chronic upper thermal limit will be lower than that needed for an acute thermal treatment. In industrial water cooling systems this strategy is used when heated water can be re-circulated throughout a system and maintained at a constant elevated temperature (Boelman *et al.* 1997). The exposure time to achieve 100 % mortality is influenced by the ambient water temperature and temperature of the treatment (i.e. when the difference between the two is lower, the exposure time needs to be prolonged) (McMahon and Ussery 1995). For example, at a temperature equal to or > 34 °C, the time necessary to achieve 100 % zebra mussel mortality can vary from 6 to 26 hours depending on the prior acclimation temperature (McMahon and Ussery 1995). Treatment temperatures ranging from 34–37 °C for industrial cooling water system are considered cost-effective and low enough to meet regulatory discharge requirements (McMahon and Ussery 1995).

There are limited data available concerning the time and temperature requirements needed to ensure complete mortality in complex marine biofouling assemblages. Thus, the number of studies specifically focusing on the application of thermal treatments on vessel sea chests and internal pipework biofouling is even smaller (Leach 2011; Piola and Hopkins 2012).

Piola and Hopkins (2012) assessed the efficacy of thermal treatment on 17 representative species typical to sea chest biofouling via laboratory experiments followed by exposure

experiments within a replica sea chest. In the laboratory experiments 100 % mortality was achieved across all three treatments (37.5 °C for 60 minutes, 40 °C for 30 minutes and 42.5 °C for 20 minutes) for the majority of species, except for the barnacle *Elminius modestus* and the oyster *C. gigas*. The trial of these temperatures within the replica sea chest produced variable results. Leach (2011) exposed biofouling species (*M. edulis* and *Trichomya hirusta*) to thermal treatments of higher temperatures and shorter intervals within a replica sea chest. 100 % mortality was achieved at a temperature of 60 °C with an exposure time of 10 minutes.

These two studies highlight several challenges that need to be addressed regarding the efficacy of thermal treatment. Both failed to achieve a uniform heat distribution throughout all areas of the replica sea chests, and for Piola and Hopkins (2012) this resulted in variable rates of mortality that were related to the position of the test organisms within the sea chest. To ensure the efficacy of thermal treatments it is imperative that an elevated water temperature occurs throughout the entire internal area of the sea chest and pipework and is maintained for the required duration.

Results from studies that test the thermal tolerance of organisms in isolation are not likely to be applicable to complex mature biofouling assemblages. Mature biofouling consisting of many species may be more tolerant to thermal treatment as the biogenic structure may provide areas of thermal refuge for some organisms. To ensure that a uniform heat distribution has been achieved, higher temperatures and longer exposure times may be required.

Temperature maintenance is predominately an engineering issue, and ensuring that a heating unit can adequately heat water within real-world sized sea chests and internal pipework is an important consideration. Leach (2011) used the patented Hull Surface Treatment™ owned by Commercial Diving Services Pty Ltd (Australia). This system consists of an applicator which covers the grate and isolates the sea chest from the external environment. Attached to the applicator is a pipe which delivers the heated water to the internal sea chest cavity. In this study the maximum flow rate of the system was 38 L per minute of heated water. Leach (2011) alluded to the development of a new pump that was capable of delivering a flow rate of 120 L per minute at a temperature of 98 °C and this would be expected to improve the performance of the thermal system (e.g. target temperature reached faster and more easily maintained).

It is likely that the water that is pumped into a sea chest will need to be at a temperature higher than that required to achieve 100 % mortality to provide confidence that the lethal temperature has been achieved throughout the system. This is due to the uneven heating of water that may occur due to the complex structures of sea chests and the difficulty in achieving an even treatment temperature throughout an established biofouling assemblage. To heat the entire internal surface area of a vessel may require multiple thermal injection points with temperature measurements made throughout the system to verify that the required maximum temperature has been achieved. Isolating the water in the sea chest and internal pipework through the application of an external water-tight cover would increase the heating efficiency and prevent mobile organisms and propagules from escaping into the environment (Leach 2011; Piola and Hopkins 2012).

Sea chest treatment studies also highlight the risk of exposing organisms to a sub-lethal thermal treatment which may result in an increase in thermal tolerance to subsequent treatments and the unintended transport of more resilient individuals (Piola and Hopkins

2012). *C. gigas* was able to survive temperatures of 43–44 °C for one hour when previously exposed to 37 °C for one hour. Further, increased thermal tolerance can be retained for at least two weeks (Clegg *et al.* 1998). Sub-lethal temperature exposure may cause some biofouling species to spawn and this could especially occur when biofouling is positioned far from the thermal injection point. Spawning has been observed to occur in mussel species (e.g. *Mytilus galloprovincialis*) that have been exposed to a rapid change in temperature (Apte *et al.* 2000). This problem could potentially be avoided by isolated the treated water.

When testing the efficacy of thermal treatments, the geographic origin of the biofouling would be expected to influence thermal tolerances, with tropical species likely to be more tolerant than temperate ones. Different populations of the same species will also be likely to display varying tolerances, especially those which occur over a wide latitudinal range or inhabit different habitat types (e.g. intertidal vs. subtidal). When conducting testing, it would be prudent to select representative taxa from intertidal habitats, as these organisms are likely to be the most thermally tolerant (Piola and Hopkins 2012).

The application of steam represents another in-water thermal treatment option. In order to apply a stream treatment, the sea chest has to be isolated with a water-tight cover to allow the seawater to be evacuated from the internal cavity. The steam can then be injected into the sea chest and internal pipework through a long hose that is connected to a steam generator (Rob Hilliard pers. comm.). Applying a steam treatment is faster and requires less energy than other hot water treatments because it avoids the energy-intensive process of heating seawater. A further advantage is that propagule release from stressed organisms will not occur due to the absence of water. The temperature applied in the situation described by Hilliard (pers. comm.) is 60 °C for 1 hour which, according to Leach (2011) and Piola and Hopkins (2012), would be expected to kill all exposed organisms. Temperature measurements of various external steel surfaces throughout the sea chest and internal pipework network will provide a level of confidence that the heat from the steam is evenly distributed. To avoid damage to paint coatings and seals, the external steel temperatures would need to be maintained below 65 °C.

6.2.4. Cost

There are no commercial heat treatment systems currently available for use on vessel niche areas (Inglis *et al.* 2012). Treatment of sea chests using the Hull Surface Treatment system on a 50, 100 and 200 m vessel were estimated to cost approximately NZ$ 5 200, $ 6 500 and $ 7 800, respectively (Inglis *et al.* 2012).

6.2.5. Category summary

6.2.5.1. Biosecurity risks

The following biosecurity risks have been identified as associated with thermal in-water treatment of sea chests and internal pipework:

- material may be dislodged from the sea chest grates by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system or equipment used to blank off the sea chests,
- containment associated with the initial application of treatment to achieve the target temperature,
- containment associated with equipment used to blank off the sea chests, and
- efficacy of the system treating the biofouling.

6.2.5.2. Feasibility

The use of thermal systems for the treatment of pipework is widespread within land-based industrial processes. Such systems have also applied to reactively treat biofouling in sea chests and internal pipework. It is therefore appropriate to develop performance standards and testing requirements for thermal in-water systems to reactively treat biofouling in sea chests and internal pipework.

Table 4: Examples of temperature and exposure times for thermal treatment of marine macro-fouling species.

Organism type	Species name	Size (mm)	Acclimation temperature (°C)	Rate of heating (°C / minute unless stated)	Treatment temperature (°C)	Mortality (% unless stated)	Time (hours)	Reference
Mussels	*Perna viridis*	2–110	29	0.1	39, 42, 44, 46	100	3.5, 0.93, 0.28, 0.15	Rajagopal *et al.* (1995b)
	P. viridis	30–48	25	0.2, 0.5, 0.8	40.8, 41.8, 43.1	100	N/A	De Bravo *et al.* (1998)
	Perna perna	30–48	25	0.2, 0.5, 0.8	38.3, 39.6, 40.5	100	N/A	De Bravo *et al.* (1998)
	Perna canaliculus	10–80	15–25.5	N/S[23]	≥ 40	100	0.08	Piola and Hopkins (2012)
	Perna indica	8–36	29	0.1	43, 44	100	14, 5	Rajagopal *et al.* (1995c)
	Mytilus galloprovincialis	10–80	15–25.5	N/S	≥ 40	100	0.33	Piola and Hopkins (2012)
	Mytilopsis leucophaeata	10	20	0.1	36	100	3.55	Rajagopal *et al.* (2005d)
	Mytilus edulis	5–30	N/S	0.6	32, 36, 40.5	95	19.8, 3.2, 0.24	Graham *et al.* (1975)

[23] Not stated

Organism type	Species name	Size (mm)	Acclimation temperature (°C)	Rate of heating (°C / minute unless stated)	Treatment temperature (°C)	Mortality (% unless stated)	Time (hours)	Reference
Mussels	*M. edulis*	40–70	N/S	2.8	32, 36, 40.5	95	22.3, 2.6, 0.26	Graham *et al.* (1975)
	M. edulis	N/S	N/S	N/S	60, 70	100	0.25, 0.16	Leach (2011)
	M. edulis	10	20	0.1	36	100	1.4	Rajagopal *et al.* (2005d)
	Trichomya hirusta	N/S	N/S	N/S	60, 70	100	0.25, 0.16	Leach (2011)
	Brachidontes striatulus	11–15	28	0.1	39, 45	100	30.2, 0.28	Masilamoni *et al.* (2002)
Oyster	*Crassostrea madrasensis*	64	30	0.1	39, 45	100	5.42, ~ 0.66	Rajagopal *et al.* (2003a)
Barnacles	*Megabalanus tintinnabulum*	5–30	28–30	0.16	37, 40, 47	100	3.37, 2.6, 0.16	Sasikumar *et al.* (1992)
	M. tintinnabulum	N/S	N/S	0.6	32, 36, 40.5	95	5.6, 0.4, instant	Graham *et al.* 1975
	Elminius modestus & Epopella plicata	N/S	15–25	N/S	42	100	0.33	Piola & Hopkins (2012)

Organism type	Species name	Size (mm)	Acclimation temperature (°C)	Rate of heating (°C / minute unless stated)	Treatment temperature (°C)	Mortality (% unless stated)	Time (hours)	Reference
Crabs	*Cancer pagurus*	80–100 (carapace width)	8, 15, 22	0.2	~ 32 (CTMax)[24]	N/A[25]	N/S	Cuculescu *et al.* (1998)
	Carcinus maenas	60–70 (carapace width)	8, 15, 22	0.2	~ 36 (CTMax)	N/A	N/S	Cuculescu *et al.* (1998)
Fishes	*Bathygobius fuscus* and *Bathygobius* spp	N/S	26	0.31	43–45 (CTMax)	N/A	N/S	Eme and Bennett (2009)
Hydroids	*Cordylophora caspoa*	N/S	19.4	0.13 (2 °C increase every 15 min)	37.7	100	1	Folino-Roerm and Indelicato (2005)
	Syncoryne eximia	N/S	N/S	0.6	32, 36, 40.5	95	0.2, instant, instant	Graham *et al.* (1975)

[24] Critical thermal maxima (CTMax) is the last temperature at which a crab was able to right itself within 1 minute after being turned over, or for fish the temperature at which the maintenance of a dorso-ventral orientation is not possible for at least one minute.
[25] Not applicable, see definition of CTMax.

6.3. Deoxygenation

6.3.1. Introduction

The reduction of dissolved oxygen in seawater can be used to treat biofouling. This has previously been achieved through the encapsulation of vessel hulls with impermeable plastic (Inglis *et al.* 2012). Once a body of water is isolated, the dissolved oxygen will be consumed by the biofouling organisms until anoxic conditions prevail. The timing of mortality is species-dependent (Inglis *et al.* 2012). Encapsulation techniques can treat the general hull area in addition to niche areas such as sea chests and internal pipework.

6.3.2. Environmental considerations

When using this treatment it is important to not dislodge biofouling when applying the plastic wrap or water-tight cover over sea chests. Also, once the treatment has been applied, the isolated water body may contain larval stages or spores that are resistant to anoxic conditions, as well as increased concentrations of chemicals (from the antifouling coating) that may require treatment before discharge into the environment.

6.3.3. Efficacy of treatment

The IMProtecter™ is a mobile encapsulation tool which is deployed around a docked vessel. For a 15 m long vessel this can occur in < 45 minutes (Floerl *et al.* 2010). This is, however, a best case scenario and it can take significantly longer to encapsulate a vessel if it is larger, being deployed in sub-optimal weather conditions (e.g. strong tidal currents, windy), or the vessel hull is irregular in shape (Justin MacDonald pers. comm.). Presently, vessels up to 18 m long and with a 5 m draft can be accommodated. Larger vessels should be capable of being treated in the future (Morrisey and Woods 2015).

Initial trials using the IMProtecter suggest that anoxia lethal to all fouling organisms can occur within 9 days in the absence of oxygen-depleting chemicals (Floerl *et al.* 2010). Although, Atalah *et al.* (2016) found that complete mortality was not achieved after 14 days with biofouling encapsulated on settlement plates.

The time to induce mortality is reliant on deoxygenation. Inglis *et al.* (2012) has estimated it will take a minimum of two days to treat a vessel via encapsulation with the addition of an oxygen scavenging chemical (see Miscellaneous treatments section), although this is likely to be dependent on the level of fouling present and water temperature which influences respiration rates (Artigaud *et al.* 2014). Bivalves and barnacles are likely to survive for prolonged periods by closing their shells and surviving in an anaerobic physiological state (Wang and Widdows 1993; Inglis *et al.* 2012). The advantage of this technique when treating sea chests and internal pipework is that any sized vessel can conceivably be treated, and once the sea chest is encapsulated all biofouling is contained (Inglis *et al.* 2012). Anoxic seawater has been shown to exhibit lower rates of corrosion on ballast water tanks (Tamburri *et al.* 2002), although corrosion may be accelerated in anoxic water through the growth of sulphate-reducing bacteria (Lee *et al.* 2005). If using an oxygen scavenging chemical it will be important to ensure that it is distributed evenly throughout the sea chest and pipework to ensure complete mortality.

6.3.4. Cost

For vessels with a turnaround time of < 4 days, this treatment would likely cause significant delays (Inglis *et al.* 2012). Commercial vessels normally have short port residence times, so this treatment (without the aid of oxygen scavenging chemicals) would impose a significant economic cost.

6.3.5. Category summary

6.3.5.1. Biosecurity risks

The following biosecurity risks have been identified as associated with in-water deoxygenation of sea chests and internal pipework:

- material may be dislodged from the general hull area or sea chest grates by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system or equipment used to encapsulate the vessel or to blank off the sea chests,
- containment associated with the initial application of treatment to achieve the target concentration,
- containment associated with equipment used to encapsulate the vessel or to blank off the sea chests, and
- efficacy of the system treating the biofouling.

6.3.5.2. Feasibility

The use of deoxygenation systems is not widespread for treatment of vessels or land-based industrial processes due to the required treatment duration. Given the short residence times of some vessel types (i.e., commercial vessels) in port, it is not appropriate to develop performance standards and testing requirements for in-water deoxygenation systems to reactively treat biofouling in sea chests and internal pipework in this context.

6.4. Freshwater (osmotic shock)

6.4.1. Introduction

Modifying the salinity of seawater through the addition of freshwater can induce osmotic shock in marine organisms. This occurs when chemical compounds passively move across semi-permeable membranes from areas of high to low concentration until an equilibrium is achieved (Reid 2012). Some marine organisms are adapted to live within a narrow salinity range and some invertebrates and macroalgae can be killed when exposed to freshwater for < 24 hours (Jones and Little 1990; Forrest and Blackmore 2006). Soft-bodied marine organisms are more likely to be susceptible to changes in salinity compared to bivalves, particularly if they are recruited from intertidal habitats (Chou *et al.* 1999).

6.4.2. Environmental considerations

If biofouling is exposed to freshwater it can cause some organisms to spawn. To reduce the likelihood of these organisms establishing it would be advisable to isolate the water body within a sea chest and internal pipework so that the larvae and gametes will not enter the environment (Inglis *et al.* 2012).

6.4.3. Efficacy of treatment

Brock *et al.* (1999) investigated biofouling survival on the Navy vessel *USS Missouri* after it remained in the Columbia River in Oregon for 9 days. After this immersion period and voyage to Hawaii it was found that 90 % of the hull was clear of fouling. In total, 11 species were found alive on the hull, of which 4 were attributed to the original biofouling. One of the surviving species found on this vessel was the mussel *Mytilus galloprovincialis*, which was found to be spawning within two hours of arrival in Pearl Harbour, although they failed to establish (Apte *et al.* 2000; Carlton and Eldredge 2015). In a separate study, two heavily fouled vessels had their biofouling assemblages surveyed before and after the 7 day transit through the freshwater Panama Canal. A total of 9 out of 22 taxa identified survived transit, with several being present in large numbers (Davidson *et al.* 2008b). Davidson *et al.* (2009) experimentally exposed marine fouling assemblages attached to PVC panels to a freshwater treatment for 12 hours. Although mortality was not 100 % there was a reduction in species richness and abundance compared to the control panels. All these studies show that the supply of propagules to recipient ports can be reduced after exposure to freshwater, although under these scenarios 100 % mortality is not achievable.

Some bivalve species are especially resilient to changes in salinity due to the ability to isolate their soft tissue from the external environment. For example, the time required for 100 % mortality of *M. californianus* and *M. edulis* following freshwater immersion was 6 and 14 hours, respectively (Fox and Corcoran 1957). Lewis and Dimas (2007) observed no mortality of *M. galloprovincialis planulatus* following exposure to freshwater for 6 and 14 hours, respectively.

6.4.4. Cost

For osmotic shock to be effective, isolation of the biofouling and extended periods of exposure are required. The advantages of this treatment are its low cost and the absence of harmful chemicals. Inglis *et al.* (2012) has suggest that vessels may have to be exposed to freshwater for 7–14 days to kill all marine biofouling. Commercial vessels have short port residence times, and osmotic shock would impose significant economic costs through disrupted itineraries.

6.4.5. Category summary

6.4.5.1. Biosecurity risks

The following biosecurity risks have been identified as associated with in-water treatment of sea chests and internal pipework with freshwater:

- material may be dislodged from the general hull area or sea chest grates by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system or equipment used to encapsulate the vessel or to blank off the sea chests,
- containment associated with the initial application of treatment to achieve the target concentration,
- containment associated with equipment used to encapsulate the vessel or to blank off the sea chests, and
- efficacy of the system treating the biofouling.

6.4.5.2. Feasibility

The use of freshwater systems is not widespread for treatment of vessels or land-based industrial processes due to the required treatment duration. Given the short residence times of some vessel types (i.e., commercial vessels) in port, it is not appropriate to develop performance standards and testing requirements for freshwater systems to reactively treat biofouling in sea chests and internal pipework in this context.

7. Miscellaneous systems

7.1. Co-treatments

7.1.1. Introduction

The application of multiple treatments simultaneously can improve performance compared to when treatments are used in isolation. This can result in enhanced efficacy or reduced exposure times.

7.1.2. Environmental considerations

The use of co-treatments will depend on their ability to induce accelerated mortality compared to using a treatment in isolation. The advantage of using this strategy is that biocides can be used at lower concentrations, lessening the regulatory burden associated with discharge limits (e.g. reduced chlorine concentration when used at an elevated temperate).

7.1.3. Efficacy of treatment

Venhuis and Rajagopal (2010) tested the ability of carbon dioxide and chlorine to induce mortality in the mussel *Mytilopsis leucophaeata*. The time to 100 % mortality when using chlorine alone at 1 mg/L was 46 days when acclimatised to 20 °C (Rajagopal *et al.* 2003b). With the addition of carbon dioxide (resulting in a reduction of the pH to 5), 100 % mortality was achieved in 6 days (Rajagopal *et al.* 2012). Jenner and Polman (2003) showed similar results when treating the marine mussel *Mytilus edulis*. The use of carbon dioxide as a pre-treatment overcomes the valve closing response of bivalves, resulting in exposure of the organism's soft tissue (Venhuis and Rajagopal 2010).

Harrington *et al.* (1997) tested the synergistic effect of chlorine and heat on the mortality rate of zebra mussels. At 30 °C and 0.5 mg/L chlorine, 95 % mortality was achieved in 1 day, while at 34 °C it was achieved in 1 hour. Compared to heat alone (30 °C), the co-treatment reduced the time required to achieve 95 % mortality by 95 %. These results are similar to Rajagopal *et al.* (2002). For *M. leucophaeata*, exposure to 0.5 mg/L chlorine at 5 °C required 99 days to achieve 95 % mortality. The required exposure time was reduced to 47 days at 30 °C. In both of these studies it was found that the synergic effect between heat and chlorine diminishes at 35–36 °C where mortality rates become similar to that obtained for heat alone.

Hydrogen peroxide and iron has been tested in combination on zebra mussels. At 5 mg/L hydrogen peroxide and 1.25 mg/L iron, 30 % mortality occurred after 56 days at a temperature of 11 °C. The long exposure required was attributed to the low water temperature (Klerks and Fraleigh 1990).

Encapsulation and the creation of anoxic conditions can be accelerated through the addition of oxygen-depleting chemicals such as nitrogen (Tamburri *et al.* 2001; Morrisey and Woods 2015). For example, sodium hydrogen sulphate ($NaHSO_4$) was added to an intake pipe to decrease the dissolved oxygen concentration in a drinking water facility in France that was fouled with zebra mussels (*Dreissena polymorpha*). This trial was not successful, however, as not all mussels were killed because it took 2 weeks for the oxygen to be consumed within the long pipe (Rajagopal *et al.* 2012). Johnson and McMahon (1998) tested the tolerance of the Asian clam (*Corbicula fluminea*) and zebra mussel to prolonged hypoxic conditions and found that tolerance to hypoxia was correlated to water temperature. For zebra mussels at a 5 % dissolved oxygen saturation level, the time required to kill 50 % of individuals was 44 days at 5 °C, 25 days at 15 °C, and 5 days at 25 °C. For the Asian clam it was 20 days at 5 °C and 6 days at 25 °C. These findings are also supported by Claudi and Mackie (1993) who found that if temperatures are low enough, zebra mussels can survive for up to 2 weeks in anoxic water. These examples highlight that methods used to reduce dissolved oxygen levels need extended exposure periods to work, especially at cooler water temperatures which result in lower respiration rates (Rajagopal *et al.* 2012). Clearwater *et al*. (2008) has recommended the use of sodium sulphite for reducing dissolved oxygen levels to control freshwater pests in New Zealand. This is because it is easy to apply and has a low toxicity to humans. Sodium sulphite combines with oxygen to produce sodium sulphate, with no by-products or change in pH (Clearwater *et al.* 2008). In addition, common ingredients such as sugar, molasses, whole milk or lactose can be added to water to increase the respiration of microorganisms and accelerate the formation of anoxic conditions (Clearwater *et al.* 2008). These ingredients are cheap to use, and their effect on the system would be easy to monitor through the measurement of dissolved oxygen (Morrisey and Woods 2015).

The manufacturer of BioBullets state their product will display increased toxicity to marine filter-feeding organisms at elevated temperatures (David Aldridge pers. comm.). No data were provided, but it would be expected that the increase in physiological activity relative to water temperature would increase the uptake of the coated biocide. Although, as was observed for chlorine and heat co-treatment, the synergistic effect is likely to diminish as the water temperature reaches 35–36 °C.

ACTI-Brom® is a proprietary bromine co-treatment used to treat zebra mussels (Sprecher and Getsinger 2000). At 0.1 mg/L, 90-100 % mortality can be achieved within 30 days at temperatures above 20 °C (Sawyko 1994).

Bracken *et al.* (in press) exposed *Mytilus galloprovincialis* to three different descalers (major active constituent in each being hydrochloric acid – HCl, acid-surfactant or phosphoric acid) at an elevated temperature of 26 °C. Dissolution of calcium carbonate shells occurred most rapidly when using the HCl descaler, with 100 % mortality occurring after 8 hours.

7.1.4. Cost

A potential limitation of co-treatments is their cost, effectiveness and the potential requirement for additional equipment. Although, the cost is likely to be variable and dependent on the co-treatments being investigated.

7.2. Category summary

7.2.1. Biosecurity risks

The following biosecurity risks have been identified as associated with in-water treatment of sea chests and internal pipework using a co-treatment approach:

- material may be dislodged from the sea chest grates by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system or equipment used to blank off the sea chests,
- containment associated with the initial application of treatment to achieve the target concentration,
- containment associated with equipment used to blank off the sea chests,
- efficacy of the system treating or removing the biofouling,
- capture of waste material removed (where removal is undertaken),
- filtration of captured waste (where removal is undertaken) and how effectively, and to what minimum particle size, material is removed from the effluent stream, and
- treatment of effluent (where removal is undertaken, for example, with heat, ultra-violet light or biocides) or discharged to a sewerage system with secondary treatment.

7.2.2. Feasibility

The use of reactive co-treatment systems shows promise for minimising the risk associated with biofouling in sea chests and internal pipework. It is therefore appropriate to develop performance standards and testing requirements for these systems.

8. Summary

This document has identified the current and emerging in-water systems that are likely to be capable of removing or treating biofouling in vessel sea chests and internal pipework in an operationally acceptable timeframe. The following broad categories are:

- chemical,
- non-chemical, and
- co-treatments.

Within these categories exist system types that warrant the development of testing frameworks and performance standards. These include: application of oxidising and non-oxidising chemicals, physical removal, thermal treatment, and co-treatments.

For each of these systems the following biosecurity risks have been identified and these will inform the development of the testing frameworks and performance standards:

- material may be dislodged from the sea chest grates by the diver's movement and equipment (fins, surface-supply air hoses, etc.) and by the in-water system or equipment used to blank off the sea chests,
- containment associated with the initial application of treatment to achieve the target concentration (where biocides are used),
- containment associated with equipment used to blank off the sea chests,
- efficacy of the system treating or removing the biofouling,
- capture of waste material removed (where removal is undertaken),

- filtration of captured waste (where removal is undertaken) and how effectively, and to what minimum particle size, material is removed from the effluent stream, and
- treatment of effluent (where removal is undertaken, for example, with heat, ultra-violet light or biocides) or discharged to a sewerage system with secondary treatment.

9. Acknowledgements

The authors would like to thank the numerous people who provided advice and guidance for this document. The authors specifically thank Stephen Cobb (MPI), Sonia Gorgula (Australian Department of Agriculture and Water Resources), Dr Justin McDonald (Western Australia Department of Fisheries), Chris Scianni (California States Lands Commission) and Kathy Walls (MPI) for their advice and peer review.

10. References

Allonier AS, Khalanski M, Camel V, Bermond A (1999). Characterization of chlorination by-products in cooling effluents of coastal nuclear power stations. *Marine Pollution Bulletin,* 38(12): 1232-1241.

Apte S, Holland BS, Godwin LS, Gardner JP (2000). Jumping ship: a stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment. *Biological Invasions,* 2(1): 75-79.

Artigaud S, Lacroix C, Pichereau V, Flye-Sainte-Marie J (2014). Respiratory response to combined heat and hypoxia in the marine bivalves *Pecten maximus* and *Mytilus* spp. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *175*: 135-140.

Atalah J, Brook R, Cahill P, Fletcher LM, Hopkins GA (2016). It's a wrap: encapsulation as a management tool for marine biofouling. *Biofouling,* 32(3): 277-286.

Bax N, Hayes K, Marshall A, Parry D, Thresher R (2002). *Man-made marinas as sheltered islands for alien marine organisms: establishment and eradication of an alien invasive marine species*. Turning the tide: the eradication of invasive species. IUCN SSC Invasive Species Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK. pp. 26-39.

Bax N, Williamson A, Aguero M, Gonzalez E, Geeves W (2003). Marine invasive alien species: a threat to global biodiversity. *Marine Policy*, 27(4): 313-323.

Bell A, Phillips S, Georgiades E, Kluza D (2011). *Risk analysis: vessel biofouling.* A report prepared by the Policy and Risk Directorate, Ministry for Primary Industries, Wellington. 155 pp.

Boelman SF, Neilson FM, Dardeau EA, Cross T (1997). *Zebra mussel (Dreissena polymorpha) control handbook for facility operators,* 1st edition. Miscellaneous paper EL-97-1. US Army Engineer Waterways Experiment Station, Vicksburg, USA. 93 pp.

Brock R, Bailey-Brock JH, Goody J (1999). A case study of efficacy of freshwater immersion in controlling introduction of alien marine fouling communities: the USS Missouri. *Pacific Science*, 53: 223-231.

Bracken J, Gust N, Ross J, Coutts A (in press). An assessment of the efficacy of chemical descalers for managing marine pests within vessel internal seawater systems and niche areas. *Management of Biological Invasions*.

Carver CE, Chisholm A, Mallet AL (2003). Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. *Journal of Shellfish Research*, 22: 621 – 631.

Carlton JT, Eldredge LG (2015*). Update and Revisions of The Marine Bioinvasions of Hawai'i: the Introduced and Cryptogenic Marine and Estuarine Animals and Plants of the Hawaiian Archipelago***.** *Bishop Museum Bulletin in Zoology*, 9: 25-47

Clegg JS, Uhlinger KR, Jackson SA, Cherr GN, Rifkin E, Friedman CS (1998). Induced thermotolerance and the heat shock protein-70 family in the Pacific oyster *Crassostrea gigas*. *Molecular Marine Biology and Biotechnology*, 7: 21–30.

Clearwater SJ, Hickey CW, Martin ML (2008). *Overview of potential piscicides and molluscicides for controlling aquatic pest species in New Zealand*. Science and Technical Publishing, Department of Conservation, New Zealand. 76 pp.

Chou CL, Zwicker B, Porter JF, Pelletier GR (1999). *Potential biofouling strategies against blue mussel (Mytilus edulis) infestation in a cooling water system*. Canadian Technical Report of Fisheries and Aquatic Sciences, 2283. 95 pp.

Chung KS, Acuna A (1981). Upper temperature tolerance limit of mussel, *Perna perna*. *Bulletin of the Japanese Society of Scientific Fisheries*, 47(3): 441.

Claudi R, Mackie GL (1993). *Practical manual for the monitoring and control of macrofouling mollusks in fresh water systems*. CRC Press. 231 pp.

Cortesia C, Vilchèze C, Bernut A, Contreras W, Gómez K, de Waard J, Jacobs WR, Kremer L, Takiff, H (2014). Acetic acid, the active component of vinegar, is an effective tuberculocidal disinfectant. *mBio* 5(2):e00013-14. doi:10.1128/mBio.00013-14.

Costa R, Moggridge GD, Aldridge DC (2012). Improved mussel control through microencapsulated BioBullets. In: Rajagopal S, Jenner HA, Venugopalan VP (Eds.) *Operational and environmental consequences of large industrial cooling water systems*, Springer. pp. 273-286.

Coutts ADM, Piola RF, Hewitt CL, Connell SD, Gardner JPA (2010).
Effect of vessel voyage speed on survival of biofouling organisms: implications for translocation of non-indigenous marine species. *Biofouling*, 26: 1-13.

Coutts AD, Dodgshun TJ (2007). The nature and extent of organisms in vessel sea-chests: a protected mechanism for marine bioinvasions. *Marine Pollution Bulletin*, 54(7): 875-886.

Coutts AD, Taylor MD (2004). A preliminary investigation of biosecurity risks associated with biofouling on merchant vessels in New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 38(2): 215-229.

Cranfield HJ, Gordon DP, Willan RC, Marshall BA, Battershill CN, Francis MP, Nelson WA, Glasby CJ, Read GB (1998). *Adventive marine species in New Zealand*. NIWA Technical Report 34. 48 pp.

Cristiani P (2005). Solutions to fouling in power station condensers. *Applied Thermal Engineering*, 25(16): 2630-2640.

Cuculescu M, Hyde D, Bowler K (1998). Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. *Journal of Thermal Biology*, 23(2): 107-110.

Davidson IC, McCann LD, Sytsma MD, Ruiz GM (2008a). Interrupting a multi-species bioinvasion vector: the efficacy of in-water cleaning for removing biofouling on obsolete vessels. *Marine Pollution Bulletin*, 56(9): 1538-1544.

Davidson IC, McCann LD, Fofonoff PW, Sytsma MD, Ruiz GM (2008b). The potential for hull-mediated species transfers by obsolete ships on their final voyages. *Diversity and Distributions*, 14(3): 518-529.

Davidson I, Sytsma M, Ruiz G (2009). *An experimental analysis of salinity shock on biofouling communities: a pilot study*. A report prepared for the California States Land Commission Marine Invasive Species Program. 22 pp.

De Bravo MS, Chung KS, Pérez JE (1998). Salinity and temperature tolerances of the green and brown mussels, *Perna viridis* and *Perna perna* (*Bivalvia*: *Mytilidae*). *Revista de Biologia Tropical*, 46 (5): 121-125.

Denny CM (2008). Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. *ICES Journal of Marine Science: Journal du Conseil*, *65*(5): 805-810.

Dore M (1989). Chimie des oxydants et traitement des eaux. Lavoisier, Paris, France. In: Rajagopal S, Jenner HA, Venugopalan VP (Eds.) *Operational and environmental consequences of large industrial cooling water systems*, Springer. pp. 227-271.

Dvorak G (no date). Disinfection 101. The Center for Food Security and Public Health. [Online] Available from: http://www.cfsph.iastate.edu/Disinfection/Assets/Disinfection101.pdf [Accessed 16th February 2016].

Eldredge LG, Carlton JT (2002). Hawai'ian marine bioinvasions: a preliminary assessment. *Pacific Science*, 56: 211-212.

Electric Power Research Institute (1993). *Hazard identification of commercially available biocides to control zebra mussels and Asiatic clams*. EPRI TR-103175. 165 pp.

Eme J, Bennett WA (2009). Critical thermal tolerance polygons of tropical marine fishes from Sulawesi, Indonesia. *Journal of Thermal Biology*, 34: 220–225.

Ferrate Treatment Technologies (2014). [Online] Available from: http://www.ferratetreatment.com/ [Accessed 26th January 2015].

Floerl O, Peacock L, Seaward K, Inglis G (2010). *Review of biosecurity and contaminant risks associated with in-water cleaning.* A report commissioned by the Department of Agriculture, Fisheries and Forestry (DAFF). Commonwealth of Australia. 136 pp.

Floerl O, Inglis GJ, Hayden BJ (2005). A risk-based predictive tool to prevent accidental introductions of nonindigenous marine species. *Environmental Management*, 35(6): 765-778.

Floerl O, Pool TK, Inglis GJ (2004). Positive interactions between nonindigenous species facilitate transport by human vectors. *Ecological Applications*, 14(6): 1724-1736.

Fofonoff PW, Ruiz GM, Steves B, Carlton JT (2003). In ships or on ships? Mechanisms of transfer and invasion for non-native species to the coasts of North America. In: Ruiz GM, Carlton JT (Eds.) *Invasive Species: Vectors and Management Strategies*, Island Press Washington. pp. 152-182.

Folino-Rorem NC, Indelicato J (2005). Controlling biofouling caused by the colonial hydroid *Cordylophora caspia*. *Water research*, 39(12): 2731-2737.

Joint I, Callow ME, Callow JA, Clarke KR (2000). The attachment of *Enteromorpha* zoospores to a bacterial biofilm assemblage. *Biofouling*, 16: 151-158.

Forrest BM, Blakemore KA (2006). Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. *Aquaculture*, 257(1): 333-345.

Forrest BM, Hopkins GA, Dodgshun TJ, Gardner JPA (2007). Efficacy of acetic acid treatments in the management of marine biofouling. *Aquaculture*, 262(2): 319-332.

Fox, DL, Corcoran EF (1957). Thermal and osmotic countermeasures against some typical marine fouling organisms. *Corrosion*, 14: 131-132. In: Chou CL, Zwicker B, Porter JF, Pelletier GR (1999). *Potential biofouling strategies against blue mussel (Mytilus edulis) infestation in a cooling water system*. Canadian Technical Report of Fisheries and Aquatic Sciences, 2283. 95 pp.

Frey MA, Simard N, Robichaud DD, Martin JL, Therriault TW (2014). Fouling around: vessel sea-chests as a vector for the introduction and spread of aquatic invasive species. *Management of Biological Invasions*, 5(1): 21-30.

Graham JW, Moncreiff RW, Benson PH, Stock JN (1975). Heat treatment for the control of marine fouling at coastal electric generating stations. In: *OCEAN 75 Conference, IEEE*. pp. 926-930.

Grandison C, Piola R, Fletcher L (2011). *A review of marine growth protection system (MGPS) options for the Royal Australian Navy*. DSTO-TR-2631. Defence Science and Technology Organisation, Australia. 37 pp.

Harrington DK, Van Benschoten JE, Jensen JN, Lewis DP, Neuhauser EF (1997). Combined use of heat and oxidants for controlling adult zebra mussels. *Water Research*, 31(11): 2783-2791.

Hewitt CL, Campbell ML, Thresher RE, Martin RB, Boyd S, Cohen BF, Currie DR, Gomon MF, Keough MJ, Lewis JA, Lockett MM, Mays N, MacArthur MA, O'Hara TD, Poore GCB, Ross DJ, Storey MJ, Watson JE, Wilson RS (2004). Introduced and cryptogenic species in Port Phillip Bay, Victoria, Australia. *Marine Biology*, 144: 183-202.

Hewitt CL, Campbell ML (2007). Mechanisms for the prevention of marine bioinvasions for better biosecurity. *Marine Pollution Bulletin*, 55(7): 395-401.

Hewitt CL, Campbell ML (2010). *The relative contribution of vectors to the introduction and translocation of invasive marine species*. A report commissioned by the Department of Agriculture, Fisheries and Forestry (DAFF). Commonwealth of Australia. 56 pp.

Hewitt CL, Willing J, Bauckham A, Cassidy AM, Cox CM, Jones L, Wotton DM (2004). New Zealand marine biosecurity: delivering outcomes in a fluid environment. *New Zealand Journal of Marine and Freshwater Research*, 38(3): 429-438.

Holt DA, Ryan E (1997). Chlorine dioxide-adult zebra mussel eradication at the Dofasco Steel Mill. *Proceedings seventh international zebra mussel and aquatic nuisance species conference*, New Orleans, January 28-31. pp. 59-67.

Inglis GJ, Floerl O, Woods C (2012). *Scenarios of vessel biofouling risk and their management. An evaluation of options*. Technical Paper No: 2012/07 for Ministry for Primary Industries, New Zealand. 126 pp.

Inglis G, Morrisey D, Woods C, Sinner J, Newton M (2013). *Managing the domestic spread of harmful marine organisms. Part A- operational tools for management*. NIWA Technical Report CHC2012-150 for Ministry for Primary Industries, New Zealand. 166 pp.

International Maritime Organization (2005). Anti-fouling systems. International convention on the control of harmful anti-fouling systems on ships.

International Maritime Organization (2011). *Guidelines for the control and management of ships' biofouling. Annex 26. Resolution MEPC.207(62)*. [Online] Available from: http://www.imo.org/blast/blastDataHelper.asp?data_id=30766 [Accessed 21st January 2015].

Jenner HA, Polman HJG (2003). New antifouling techniques by combined carbon dioxide and sodium hypochlorite dosing. In: Mackie GL, Claudi R (Eds.) *Monitoring and control of macrofouling mollusks in fresh water systems*, 2nd edition (2009), CRC Press Inc. pp. 329-353.

Jenner HA, Whitehouse JW, Taylor CJ, Khalanski M (1998). Cooling water management in European power stations biology and control of fouling. *Hydroécologie Appliquée*, 10: 1-225.

Johnson PD, McMahon RF (1998). Effects of temperature and chronic hypoxia on survivorship of the zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*). *Canadian Journal of Fisheries and Aquatic Sciences*, 55(7): 1564-1572.

Jones JM, Little B (1990). *USS Princeton (CG 59): impact of marine macrofouling (mussels and hydroids) on failure/corrosion problems in seawater piping systems*. Naval Oceanographic and Atmospheric Research Laboratory, USA. 19 pp.

Khalanski M, Jenner HA (2012). Chlorination chemistry and ecotoxicology of the marine cooling water systems. In: Rajagopal S, Jenner HA, Venugopalan VP (Eds.) *Operational and environmental consequences of large industrial cooling water systems*, Springer. pp. 183-226.

Klerks PL, Fraleigh PC (1991). Controlling adult zebra mussels with oxidants. *Journal American Water Works Association*, 92-100. In: Petrille JR, Miller SD (2000). Efficacy of

hydrogen peroxide for control of adult zebra mussels, *Dreissena polymorpha*, and Asian clams, *Corbicula fluminea*. *10th international aquatic nuisance species and zebra Mussel conference*, Toronto, February 13-17, 2000. 9 pp.

Kitis M (2004). Disinfection of wastewater with peracetic acid: a review. *Environment International*, 30(1): 47-55.

Leach A (2011). *Testing the efficacy of heated seawater for managing biofouling in ships' sea chests*. Honours Thesis, University of Wollongong, Australia. 70 pp.

Lee JE, Chown SL (2007). *Mytilus* on the move: transport of an invasive bivalve to the Antarctic. *Marine Ecology Progress Series*, 339: 307-310.

Lee JS, Ray RI, Little BJ, Lemieux EJ (2005). Evaluation of deoxygenation as a corrosion control measure for ballast tanks. *Corrosion*, 61(12): 1173-1188.

Lehaitre M, Delauney L, Compère C (2005). Biofouling and underwater measurements. *Real-time observation systems for ecosystem dynamics and harmful algal blooms. UNESCO, Paris*. [Online] Available from: ftp://193.49.112.3/pub/marcel/Book%20Chapters/Leha%EEtre/First%20draft/Texte_HABmlhcc.pdf [Accessed 16th February 2016].

Lewis JA (2013). *In-water hull cleaning and filtration system: in-water cleaning trials*- 26-28 November 2012. Fisheries Occasional Publication, Western Australia Department of Fisheries, 114. 44 pp.

Lewis JA, Dimas J (2007). *Treatment of biofouling in internal seawater systems – phase 2*. DSTO-TR-2081. Defence Science and Technology Organisation, Australia. 25 pp.

Lewis JA, Smith BS, Taylor RJ, Batten JJ (1988). Fouling of RAN submarine systems and a comparison of electro-chemical control methods. *Proceedings of the eighth international corrosion conference, Plymouth, UK*. 20 pp.

Mackie GL, Claudi R (2009a). Domination by chlorination. In: Mackie GL, Claudi R (Eds.) *Monitoring and control of macrofouling mollusks in fresh water systems*, 2nd edition, CRC Press Inc. pp. 329-353.

Mackie GL, Claudi R (2009b). Weapons of mollusk destruction. In: Mackie GL, Claudi R (Eds.) *Monitoring and control of macrofouling mollusks in fresh water systems*, 2nd edition, CRC Press Inc. pp. 287-327.

Martin MD, Mackie GL, Baker MA (1993). Acute toxicity tests and pulsed-dose delayed mortality at 12 and 22° C in the zebra mussel (*Dreissena polymorpha*). *Archives of Environmental Contamination and Toxicology*, 24(3): 389-398. In: Chou CL, Zwicker B, Porter JF, Pelletier GR (1999). *Potential biofouling strategies against blue mussel (Mytilus edulis) infestation in a cooling water system*. Canadian Technical Report of Fisheries and Aquatic Sciences. 89 pp.

Masilamoni JG, Nandakumar K, Jesudoss KS, Azariah J, Satapathy KK, Nair KVK (2002). Influence of temperature on the physiological responses of the bivalve *Brachidontes*

striatulus and its significance in fouling control. *Marine Environmental Research*, 53(1): 51-63.

Matisoff G, Brooks G, Bourland IB (1996). Toxicity of chlorine dioxide to adult zebra mussels. *American Water Works Association*, 88(8): 93-106.

McMahon RF, Ussery TA (1995). *Thermal tolerance of zebra mussels (Dreissena polymorpha) relative to rate of temperature increase and acclimation temperature*. Technical Report EL-95-10. US Army Engineer Waterways Experiment Station, Vicksburg, USA. 28 pp.

Montanat M, Merle G, Migeon B (1980). *Essai d'utilisation du dioxyde de chlore dans la boucle TERA installee a Montereau. Resultats des tests de toxicite*. EDF DER Report HEI31-80.044. In: Jenner HA, Whitehouse JW, Taylor CJ, Khalanski M (1998). Cooling water management in European power stations biology and control of fouling. *Hydroécologie Appliquée*, 10: 1-225.

Morrisey D (2015). *Addition of biocide during vessel biofouling treatment – an assessment of environmental effects*. Prepared for Nelson City Council. Cawthron Report No. 2715. 72 pp.

Morrisey D, Gadd J, Page M, Floerl O, Woods C, Lewis J, Bell A, Georgiades E (2013). *In-water cleaning of vessels: Biosecurity and contamination risk*. Prepared for the Ministry of Primary Industries, New Zealand. 265 pp.

Morrisey D, Woods C (2015). *In-water cleaning technologies: Review of information*. Prepared for the Ministry for Primary Industries, New Zealand. 53 pp. [Online] Available from: http://www.mpi.govt.nz/document-vault/10814 [Accessed 25th February 2016].

Morrisey DJ, Depree CV, Hickey CW, McKenzie DS, Middleton I, Smith MD, Stewart M, Thompson, KJ (2016). Rapid treatment of vessels fouled with an invasive polychaete, *Sabella spallanzanii*, using a floating dock and chlorine as a biocide. *Biofouling*, 32(2), 135-144.

Neil KM, Stafford H (2005). *Efficacy of treatments against internal biofouling in small vessels*. Queensland Department of Primary Industries and Fisheries and CRC Reef, report to the Australian Government Department of Agriculture, Fisheries and Forestry, 30 pp.

Ocean Portal (2015). Ocean Acidification. Smithsonian National Museum of Natural History. [Online] Available from: http://ocean.si.edu/ocean-acidification [Accessed 22nd January 2015].

Otani M (2006). Important vectors for marine organisms unintentionally introduced to Japanese waters. In: Koike F, Clout MN, Kawamichi M, De Poorter M, Iwatsuki K (Eds.) *Assessment and control of biological invasion risks*, IUCN, Switzerland. pp. 92-103.

Oxford dictionaries (2015). Osmosis. [Online] Available from: http://www.oxforddictionaries.com/definition/english/osmosis [Accessed 10th March 2015].

Palermo M (1992). *Components of vessels and dredges susceptible to zebra mussel infestations*. Technical Note ZMR-3-07. US Army Engineer Waterways Experiment Station, Vicksburg, USA. 3 pp.

Perepelizin PV, Boltovskoy D (2011). Hot water treatment (chronic upper lethal temperature) mitigates biofouling by the invasive Asian mussel *Limnoperna fortunei* in industrial installations. *Environmental Science and Technology*, 45(18): 7868-7873.

Petrille JR, Miller SD (2000). Efficacy of hydrogen peroxide for control of adult zebra mussels, *Dreissena polymorpha*, and Asian clams, *Corbicula fluminea*. *10th international aquatic nuisance species and zebra mussel conference*, Toronto, February 13-17, 2000. 9 pp.

Piola RF, Dunmore RA, Forrest BM (2009). Assessing the efficacy of spray-delivered 'eco-friendly 'chemicals for the control and eradication of marine fouling pests. *Biofouling*, 26(2), 187-203.

Piola R, Grandison C (2013). *In-water treatment of biofouling in internal systems: field validation of quarternary ammonium compound (QAC) chemical treatment protocols*. DSTO-TR-2774. Defence Science and Technology Organisation, Australia. 39 pp.

Piola R, Hopkins G (2012). Thermal treatment as a method to control transfers of invasive biofouling species via vessel sea chests. *Marine Pollution Bulletin*, 64: 1620-1630.

Reid DF (2012). *The role of osmotic stress (salinity shock) in protecting the Great Lakes from ballast-associated aquatic invaders.* Technical report prepared for the U.S. Saint Lawrence Seaway Development Corporation, United States Department of Transportation. 39 pp.

Rajagopal S (2012). Chlorination and biofouling control in industrial cooling water systems. In: Rajagopal S, Jenner HA, Venugopalan VP (Eds.) *Operational and environmental consequences of large industrial cooling water systems*, Springer. pp. 163-182.

Rajagopal S, Jenner HA, Venugopalan VP, Khalanski M (2012). Biofouling control: alternatives to chlorine. In: Rajagopal S, Jenner HA, Venugopalan VP (Eds.) *Operational and environmental consequences of large industrial cooling water systems*, Springer. pp. 227-271.

Rajagopal S, Van der Velde G (2012). Invasive species: implications for industrial cooling water systems. In: Rajagopal S, Jenner HA, Venugopalan VP (Eds.) *Operational and environmental consequences of large industrial cooling water systems*, Springer. pp. 127-162.

Rajagopal S, Van der Velde G, Van der Gaag M, Jenner HA (2005a). Byssal detachment underestimates tolerance of mussels to toxic compounds. *Marine Pollution Bulletin*, 50(1): 20-29.

Rajagopal S, Venugopalan VP, Van der Velde G, Jenner HA (2005b). Response of mussel *Brachidontes variabilis* to chlorination. *Chemistry and Ecology*, 21(2): 119-132.

Rajagopal S, Van der Velde G, Jansen J, Van der Gaag M, Atsma G, Janssen-Mommen JP, Jenner HA (2005c). Thermal tolerance of the invasive oyster *Crassostrea gigas*: feasibility of heat treatment as an antifouling option. *Water Research*, 39(18): 4335-4342.

Rajagopal S, Van der Velde G, Van der Gaag M, Jenner HA (2005d). Factors influencing the upper temperature tolerances of three mussel species in a brackish water canal: size, season and laboratory protocols. *Biofouling*, 21(2): 87-97.

Rajagopal S, Venugopalan VP, Van der Velde G, Jenner HA (2003a). Comparative chlorine and temperature tolerance of the oyster *Crassostrea madrasensis*: Implications for cooling system fouling. *Biofouling*, 19(2): 115-124.

Rajagopal S, Van der Velde G, Van der Gaag M, Jenner HA (2003b). How effective is intermittent chlorination to control adult mussel fouling in cooling water systems? *Water Research*, 37(2): 329-338.

Rajagopal S, Venugopalan VP, Van der Velde G, Jenner HA (2003c). Tolerance of five species of tropical marine mussels to continuous chlorination. *Marine Environmental Research*, 55(4): 277-291.

Rajagopal S, Venugopalan VP, Van der Velde G, Jenner HA (2003d). Response of fouling brown mussel, *Perna perna* (L.) to chlorine. *Archives of Environmental Contamination and Toxicology*, 44(3): 369-376.

Rajagopal S, Van der Gaag M, Van der Velde G, Jenner HA (2002). Control of brackish water fouling mussel, *Mytilopsis leucophaeata* (Conrad), with sodium hypochlorite. *Archives of Environmental Contamination and Toxicology*, 43(3): 296-300.

Rajagopal S, Nair KVK, Van der Velde G, Jenner HA (1997). Response of mussel *Brachidontes striatulus* to chlorination: an experimental study. *Aquatic Toxicology*, 39(2): 135-149.

Rajagopal S, Venugopalan VP, Nair KVK, Azariah J (1995a). Response of green mussel, *Perna viridis* (L.) to chlorine in the context of power plant biofouling control. *Marine and Freshwater Behaviour and Physiology*, 25(4): 261-274.

Rajagopal S, Venugopalan VP, Azariah J, Nair KVK (1995b). Response of the green mussel *Perna viridis* (L.) to heat treatment in relation to power plant biofouling control. *Biofouling*, 8(4): 313-330.

Rajagopal S, Nair KVK, Azariah J (1995c). Response of brown mussel, *Perna indica*, to elevated temperatures in relation to power plant biofouling control. *Journal of Thermal Biology*, 20(6): 461-467.

Rajagopal S, Van der Velde G, Jenner HA (1994). *Biology and control of brackish water mussel, Mytilopsis leucophaeata in the Velsen and Hemweg power stations, The Netherlands. Part II. Control measures*. In: Jenner HA, Whitehouse JW, Taylor CJ, Khalanski M (1998). Cooling water management in European power stations biology and control of fouling. *Hydroécologie Appliquée*, 10: 1-225.

Reid JD (1932). The disinfectant action of certain organic acids. *American Journal of Hygiene*, 16: 540-556.

Rolheiser KC, Dunham A, Switzer SE, Pearce CM, Therriault TW (2012). Assessment of chemical treatments for controlling *Didemnum vexillum*, other biofouling, and predatory sea stars in Pacific oyster aquaculture. *Aquaculture*, 364: 53-60.

Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997). Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist*, 37(6), 621-632.

Sasikumar N, Nair KVK, Azariah J (1992). Response of barnacles to chlorine and heat treatment: an experimental study for power plant biofouling control. *Biofouling*, 6(1): 69-79.

Sawyko PM (1994). Development of zebra mussel control programs utilizing chlorine, ACTI-BROM, and bromine. In: Sprecher SL, Getsinger KD (2000). *Zebra mussel chemical control guide.* Technical Report ERDC/EL TR-00-1. US Army Engineer Waterways Experiment Station, Vicksburg, USA. 105 pp.

Schultz MP, Bendick JA, Holm ER, Hertel WM (2011). Economic impact of biofouling on a naval surface ship. *Biofouling*, 27(1): 87-98.

Scianni C, Brown C, Newson A, Nedelcheva R, Falkner M, Dobroski N (2013). *2013 Biennial report on the California marine invasive species program*. A report produced for the California State Legislature by the California State Lands Commission Marine Facilities Division. 157 pp.

Sharma VK (2002). Potassium ferrate (VI): an environmentally friendly oxidant. *Advances in Environmental Research*, 6(2): 143-156.

Sprecher SL, Getsinger KD (2000). *Zebra mussel chemical control guide*. Technical Report ERDC/EL TR-00-1. US Army Engineer Waterways Experiment Station, Vicksburg, USA, 105 pp.

Tamburri MN, Wasson K, Matsuda M (2002). Ballast water deoxygenation can prevent aquatic introductions while reducing ship corrosion. *Biological Conservation*, 103(3): 331-341.

Tsolaki E, Diamadopoulos E (2010). Technologies for ballast water treatment: a review. *Journal of Chemical Technology and Biotechnology*, 85(1), 19-32.

Venkatesan R, Murthy PS (2009). Macrofouling control in power plants. In: Murthy PS, Venkatesan R, Cooksey K (Eds.) *Marine and Industrial Biofouling,* Springer. pp. 265-291.

Venhuis LP, Rajagopal S (2010). Responses (mortality and sublethal) of *Mytilopsis leucophaeata* to elevated CO_2 in combination with sodium hypochlorite. In: Rajagopal S, Jenner HA, Venugopalan VP (Eds.) *Operational and environmental consequences of large industrial cooling water systems*, Springer. pp. 227-271.

Wang WX, Widdows J (1993). Metabolic responses of the common mussel *Mytilus edulis* to hypoxia and anoxia. *Marine Ecology Progress Series*, 95, 205-214.

Woods C, Floerl O, Fitridge I, Johnston O, Robinson K, Rupp D, Davey N, Rush N, Smith M (2007). *Efficacy of hull cleaning operations in containing biological material. II. Seasonal variability*. MAF Biosecurity New Zealand, Technical Paper Series 08/11. 116 pp.