# Review of in-water hull encapsulation and enclosure treatments for eliminating marine biofouling

Prepared for the Ministry for Primary Industries
By Ulla Ammon, Luke Swift, Sarah Brand, Andrew Jeffs and Simon Swift
(The University of Auckland)

April 2019

# 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/

# Review of In-Water Hull Encapsulation
# and
# Enclosure Treatments for Eliminating Marine Biofouling

**Prepared by Ulla Ammon, Luke Swift, Sarah Brand, Andrew Jeffs and Simon Swift**
**Reviewed by Brendon Dunphy**

**EXECUTIVE SUMMARY**

In recent years, in-water hull encapsulation and enclosure systems have begun to be used for treating marine biofouling on vessel hulls. These systems are considered by some to be a potentially effective intervention for the introduction and spread of non-indigenous species (NIS) to New Zealand's marine environment via biofouling on vessel hulls. The effectiveness of these systems in treating hull biofouling relies to a large extent on the biocides that are used in the treatment, and the manner in which the biocides are applied. It is also possible to treat hull fouling using these systems without the addition of a biocides by allowing unfavourable conditions to develop in the water enclosed around the hull, but this approach to treatment takes much longer to be effective. While some biocides may be highly effective at destroying hull biofouling, they may also create problems in terms of storage, handling and disposal. Therefore, the overall aim of this report was to review the current state of knowledge of biocides that are used for in-water hull encapsulation and enclosure systems, as well as to identify other biocides with potential to be used for this application.

In New Zealand, granulated chlorine (usually sodium dichloroisocyanurate or dichlor) has become established as the predominant biocide used in encapsulation and enclosure systems. The more common use of this biocide is as much a result of convenience and experience, rather than a rigorous comparison of the various biocide options. Regardless, the New Zealand Environmental Protection Authority has issued an approval for chlorine to be used specifically for this purpose. In addition, at least three regional councils have issued resource consents for the release of neutralised chlorine by-products associated with the use of this biocide in enclosure systems. However, there is a relatively limited history of use of this biocide under New Zealand conditions for this purpose, largely because of the recent emergence of encapsulation and enclosure systems and their limited use to date. This is despite the availability of four encapsulation systems dedicated for this purpose stationed widely around New Zealand, in Northland, Nelson and Southland, as well as enclosure systems deployed by commercial diving operators. Where granulated chlorine has been used in these systems (typically >200 ppm for at least 24 hours) it has reportedly proven to be highly effective on most vessel hull biofouling, however, some more resilient taxa, such as bivalves, are able to survive treatment periods. Furthermore, it appears that it is essential that the active concentration of biocide is maintained throughout the full period of treatment, and that it is actively circulated throughout the water held within the enclosure so that it reaches all confines of the hull surface.

Enquiries through networks overseas indicate that encapsulation and enclosure systems for treating biofouling for biosecurity reasons have received minimal attention and use, and therefore are not a particularly useful source for more detailed information on biocides for this application.

A comprehensive review of the available information identified a wide range of biocides with the potential to be used to eliminate biofouling when used in conjunction with encapsulation and enclosure systems. This included both chemical and non-chemical forms of biocidal interventions. Where biocides with potential were identified by the information review, details were then sought on the varying aspects of their potential for application for treating biofouling in encapsulation and enclosure systems. These aspects included, their effectiveness against a broad range of biofouling taxa, their feasibility in terms of exposure concentration and duration, their compatibility with vessel and enclosure infrastructure, their cost, and their practicalities in terms of safety to operators and the environment. However, there is a lack of comprehensive

information for most biocides identified to have potential for this application. Often information, where available, is confined to the results of laboratory studies on specific taxa and not on an in-field application against a broad range of biofouling taxa. Furthermore, information on the efficacy of combinations of biocidal treatments is almost non-existent. As a result of this situation, only a small number of biocides appear to have sufficient information and/or a track record of use to indicate their effectiveness, for which chlorine and acetic acid are the lead contenders.

The reliable elimination of biofouling taxa with calcareous exoskeletons, such as bivalves, barnacles and some tubeworms, when exposed to biocidal treatments is the most significant challenge to the efficacy of biocides, including chlorine and acetic acid. Therefore, biocidal treatment protocols for encapsulation and enclosure systems need to be further developed, to better demonstrate their effectiveness for extended dose and duration, or by using in conjunction with other agents (such as chemical relaxants or anaesthetics), that would increase the biocidal activity against more resistant taxa. This is particularly the case for the use of chlorine which is already the preferred biocide for the treatment of biofouling in encapsulation and enclosure systems, has regulatory approval for use in New Zealand, and is safer to handle than some leading alternative biocides, i.e., strong solutions of acetic acid. There may also be the potential for different biocidal treatment protocols to be used in response to specific biofouling situations. For example, acetic acid treatment appears to have greater effectiveness against more resistant organisms with calcareous exoskeletons, such as bivalves and barnacles, because of its ability to degrade the calcareous shell material.


**INTRODUCTION**


Vessel hulls are a major vector for the introduction of marine NIS into and within new regions of the world, including New Zealand (Carlton and Geller 1993, Coutts and Taylor 2004). For example, around 90 per cent of the more than 350 NIS introduced to the marine environment in New Zealand are thought to have arrived as biofouling on vessel hulls (Piola and Conwell 2010, Statistics New Zealand 2016). Therefore, an important approach to the prevention of further NIS establishing in New Zealand waters is the interception and destruction of NIS on the hulls of vessels, especially those arriving from overseas. For example, of all the individual marine biofouling species that were sampled from the hulls of 11 fishing vessels arriving from overseas, over half the biofouling species were NIS (Piola and Conwell 2010). The Ministry for Primary Industries has long recognised the potential impact of marine NIS to the New Zealand environment and economy. As a result the Ministry and its predecessors have been at the forefront of investigating potential methods of prevention and intervention, which have been evaluated and advanced through numerous commissioned reports (e.g., Golder Associates NZ Ltd 2008, Coutts and Forrest 2005, Aquenal 2009, Piola and Conwell 2010, Inglis et al. 2012, Morrisey and Woods 2015, Growcott et al. 2016, Cahill et al. 2018).

In-water hull encapsulation and enclosure treatments for the destruction of biofouling is emerging technology for the treatment of biofouling on vessel hulls. The treatment involves fully enclosing the hull with a sheath and then either treating the residual water around the hull with a biocide, or leaving the enclosed water to become anoxic or unfavourable for biofouling, with the aim of destroying all living biofouling, including any NIS, associated with the vessel hull (Aquenal 2009, Morrisey and Woods 2015, Atalah et al. 2016). The effectiveness of these treatment systems relies heavily on the efficacy of the applied biocidal treatment. This includes both the addition of

chemical biocides, as well as using anoxia of the stagnating encapsulated water around the hull to kill biofouling without the addition of chemicals. Therefore, the overall aim of this report was to undertake an extensive review of biocides currently used, and those with the potential to be used, for in-water hull encapsulation and enclosure treatments.

The comprehensive review involved an extensive literature search using conventional information technology tools, as well as accessing professional expert networks. Specifically, the review identified the key biocides that have been used in the in-water hull encapsulation and enclosure treatment systems, and those with potential to be used. The effectiveness of these biocides was assessed in terms of active concentrations, required contact time, spectrum of activity against the range of biofouling taxa, and their ecotoxicity. A consideration of the mode of action of each biocide was undertaken to provide the basis for predictions on the spectrum of activity where the data was only available for a selected aquatic species. A consideration of the toxicity of each biocide against non-aquatic species was undertaken to provide the basis of predictions on ecotoxicity where such data was not available.

The key outcomes from the review were to develop and present the following:

• An expert evaluation of efficacy of biocides for this application, i.e., safe biocides, concentration, contact time for a variety of biofouling taxa, including both soft taxa (i.e., sea squirts, tubeworms, algae), and hardier taxa (i.e., mussels, oysters, barnacles).

• A readily accessible table summarising the efficacy of biocides for this application.

• A database of sourced literature used for the review where copyright permissions allow.

• Identification of gaps in the knowledge, e.g., biocide ecotoxicity data for aquatic species in New Zealand waters, biocide effectiveness against New Zealand invasive species.

• Consideration and evaluation of biocides which have the potential to be used for encapsulation/enclosure treatment but have yet to be tested specifically using this approach or in the field.

• A set of recommendations pertaining to the effectiveness of encapsulation for the proposed purpose and the best choices of biocide, concentration and contact time for effective encapsulation treatment.

• Identification of any areas where further research is needed.

**METHODOLOGY**

The central element of the preparation of this report was a detailed information search and review to identify material relevant to the topic.

Well recognised literature sources, such as scientific journal articles, patents, trade journal articles, were firstly identified using searches of online databases using the initial search terms (encapsulat* OR enclos*) AND (biofouling AND (biocide OR antifouling)) AND (vessel OR ship OR

4

hull OR boat OR "dry dock"). Further searches were further refined or extended as needed with additional or refined search terms. For example, focussed searches were undertaken on the use of particular biocides initially identified in the literature survey or through the prior expert knowledge of the review team.

The key databases initially searched included:-
   *ProQuest* – returning 659 documents, with 37 additional databases identified for further search
   *Scopus* -returning 144 documents
   *Reaxys Results* – returning 580 documents
   *Reaxys Patents* – returning 3 documents
   *Compendex* – returning 3 documents
   *PatentScope* – returning 943 documents
   *Derwent Patents* – returning 4 documents
   *Embase* – returning 3 documents
   *Embase Classic (Archive)* – returning 0 documents

Documents identified by database searches were examined for relevance, usually firstly by checking title, then abstract, and if assessed to be relevant the documents were recovered for further review processing. Cited references in recovered documents were back sourced and cross referenced to ensure complete coverage of literature. For example, a quite extensive body of work already conducted in this area, much of it supported by government agencies in New Zealand, provided immediate access to easily accessible literature in this field (e.g., Coutts and Forrest 2007, Atalah et al. 2016, Growcott et al. 2016, 2017, Cahill et al. 2018). In addition, the wider knowledge of the scientific and technical literature of the reviewing team was drawn upon to ensure relevance and adequacy of the information searches.

Web-based material was accessed in a similar manner to database searches, using Google searches with combinational search terms, followed by assessment of search result for relevancy, and the identification of material suitable for recovery.

Grey literature, such as internal reports and company promotional/ technical specifications, were accessed through personal contacts with international scientific, policy and industry networks of the review team for inclusion in the review. Information was also gathered through telephone, Skype and email interviews with experts, both in New Zealand and overseas, who have direct experience of vessel encapsulation and enclosure technology. In total more than 20 personnel with some knowledge in the field were contacted for information.

*EndNote* referencing management software was used to establish a reference library of written material that was assessed as being relevant to the review. Entry into *EndNote* included a reference and where possible an abstract entry, and electronic copy of the source reference document.

The relevant information recovered by the research team was interrogated to identify key biocides, and to extract key values for parameters relating to each identified biocide, such as effectiveness at varying concentrations, ecotoxicity, vulnerability of a range of biofouling taxa etc. These values were summarised into a readily accessible reference table for the review.

Further review of the data by the review team was used as the basis for the textual content of the topic review, the identification of knowledge gaps, and the formulation of conclusions and recommendations.

The draft review report was independently peer reviewed internally by a researcher who is active in the field of aquatic biocides, and further changes were made in response to the peer review, to form the basis of this final report.

## BACKGROUND

The use of in-water hull encapsulation and enclosure systems for the treatment and removal of marine biofouling on vessel hulls is a relatively new intervention for the prevention of accidental translocation and introduction of marine NIS via vessel hull biofouling (Coutts and Forrest 2005, 2007, Aquenal 2009, Roche et al 2015, Morrisey and Woods 2015, Atalah et al. 2016). These systems provide an alternative approach to treating the biofouling on vessel hulls, where hull antifouling and cleaning have failed to prevent biofouling, or where lifting the vessel out of the water for cleaning may not be practical or appropriate. In-water hull encapsulation and enclosure systems have attracted the most interest and use in Australasia, especially New Zealand.

The most highly publicised approach to in-water enclosure treatments has been the adapted use of in-water hull enclosure systems that are widely used overseas for maintaining clean hulls on vessels when the vessels are not in use, such as when berthed in marinas. These enclosure systems usually involve a floating surround for the hull that suspends a synthetic sheet which fully encloses the hull. Vessels are typically driven into the enclosure while the rear of the enclosure is submerged, allowing the vessel to pass over, and once inside the rear of the enclosure can be re-instated or inflated to facilitate the synthetic sheet completely surrounding the hull. The enclosed water can then be removed from around the hull by pumping out from the enclosure, most often via pump ports in the enclosure membrane. Typically when in-water enclosure systems are just being used for storing vessels, as much water as possible is removed from the enclosure. However, if these in-water enclosure systems are to be used for treating biofouling, some water is purposely left in the enclosure, to which biocide is added in order to kill the biofouling on the hull.

The use of an enclosure system for the treatment of hull biofouling may further reduce the risk of the release of NIS into the local environment as the enclosed system will provide continued exposure to the biocide for any taxa or larvae that detach from the vessel, which is of particular importance in preventing free spawning of biofouling organisms that have been shown to be stimulated to spawn under chemical treatments (Coutts and Dodgshun 2007, Inglis et al. 2012). Likewise, mobile marine NIS, such as crabs, gastropods (i.e., snails) or fish that may otherwise escape into the environment can be expected to be retained and exposed to the biocide within an enclosure system.

There are four enclosure systems currently in use for the treatment of hull biofouling in New Zealand, two with the Northland Regional Council, one with Top of the South's Marine Biosecurity Partnership based in Nelson, and the largest unit with the Environment Southland. The current cost of these units is around $35,000 depending on the size and specifications.

Despite owning two enclosure systems, the Northland Regional Council rarely uses the units. The small unit was used twice in the last year for treating biofouling on vessel hulls, compared to between 100 to 150 boats which were directed by Council officers to haul out yards for decontamination, as well as numerous boats which had only small amounts of biofouling of concern removed and recovered by hand by SCUBA divers. For most conventional small vessels haul out yards are generally a lower cost option than deploying in-water enclosure systems. Haul out also provides opportunity for complete containment of removed biofouling, and ready access to conduct additional observation and servicing, such as the application of antifouling and accessing difficult areas, such as around rudder and propeller fittings, as well as sea chests.

The major reason for the lack of use of the in-water enclosure systems is the cost of deploying the units, which because of their bulk usually require at least six people to work together to deploy, and then later to recover and pack down the units. For example, the smaller units weigh over 150 kg, plus additional metal pipes and chain. Once wet, the unit is heavier and more difficult to recover. However, the units are portable and can be moved on trailers, boats or helicopters, and can be easily lifted and retrieved with lifting equipment, such as a crane or Hiab. Their portability gives them the potential to be used in remote locations where haul out facilities are not readily available to deal with a crisis situation. This scenario was the major driver for Southland Regional Council purchasing their large unit in 2016, so they could respond to any vessels of concern arriving in Fiordland, which is considered an area of unique marine biodiversity. However, the unit is yet to be deployed for this purpose within the region. The Top of the South partnership purchased their unit early in 2018 and have not deployed it for a treatment situation yet, and they also report similar practical difficulties encountered during practice deployments of the unit.

All three sites using enclosure treatment systems use granulated chlorine (generally sodium dichloroisocyanurate or 'dichlor') at over 200 ppm for a minimum of four hours of exposure (Morrisey 2015), and usually overnight. A lower concentration of 50 ppm has been used for 24 hours, but is reportedly not as effective as the higher concentration, especially when the higher concentration can be left for a longer period and pumps are used to continually circulate the biocide around the enclosed hull, with the chlorine concentration being monitored with a simple test kit to ensure the concentration is maintained throughout the treatment period. Typically the concentration of active chlorine decreases during the treatment period, especially after initial application. Health and safety protocols are required for the handling of the granulated chlorine, which is usually measured into a plastic bucket and thoroughly mixed with water before being introduced as a concentrated liquid to the water enclosed against the vessel hull.

Operators of the enclosure system in Northland report that its use in this manner results in a reliable kill of biofouling of most concern to them (e.g., Mediterranean fanworm and clubbed sea squirt), with much of the biofouling dropping off the hull and into the enclosure where it can be recovered. They have not encountered damage to the vessel or enclosure material as a result of the chlorine application.

In 2016 the Environmental Protection Authority (EPA) approved chlorine as a hazardous substance for the use of treating boat hulls for removing of biofouling for biosecurity reasons (APP202796, EPA 2016). Following treatment the chlorine in the enclosure is neutralised with the addition of sodium thiosulphate and the water released to the environment under a resource consent granted for this purpose. Where used close to urban sewage reticulation and treatment plants it would

also be theoretically possible to pump out the enclosed seawater/biocide mixture and dispose of into a sewage system.

These enclosure systems, while seemingly portable, are difficult to deploy, and this is especially true for larger units. It may be possible, and preferable, to deploy the units as permanently moored fixtures in marinas to allow for the easier treatment of vessels of concern, and this would also allow for land-based treatment of biocide residues. Such an approach, described as a decontamination berth, has been tested as a prototype in Wales, UK, with some apparent success (Roche et al. 2015). Similar prototype devices for encapsulating vessels in situ have been developed (Aquenal, 2009). One of these units was purchased by Fisheries Western Australia around 5 years ago and tested on a variety of keeled yachts. However, the unit was found to be unwieldy to operate in a marina environment, and subsequent testing with hauled tarpaulins found they were easier to install to enclose yacht hulls. They relied on pumping out enclosed water and allowing anoxia to develop over at least 10 days, which was reportedly effective for killing even hardy biofouling oysters. Neither enclosure of vessels with tarpaulins or the enclosure units are now in use in Western Australia, with the agency now recommending vessels of biosecurity concern are hauled out and thoroughly cleaned.

A permanent enclosure dock could allow for larger units to be built to treat boats of hull lengths larger than is possible at present, i.e., 25 m. Most recreational vessels are small enough to fit into the existing portable enclosure units that are available around New Zealand. Furthermore, the larger units are sufficiently flexible to be able to accommodate wider multihull vessels. Alternatively, a powered dock that can be driven to treatment sites where it would allow vessels to be driven into the dock and enclosed for treatment could be used for in-water hull enclosure, with a prototype having been built and tested (Morrisey and Woods 2015).

Wrapping vessel hulls in plastic sheet or canvas material is another approach to enclosing and treating biofouling on vessels which has been deployed in New Zealand with varying success for at least 8 years (Denny 2008, Coutts and Forrest 2007, Morrisey and Woods 2015). A commercial diving company in Nelson has been a leader in developing and applying this technology, which has been effectively applied to other biofouling control situations, including aquaculture and wharf infrastructure (Coutts and Forrest, 2007, Mantelatto et al. 2015, Roche et al. 2015, Atalah et al. 2016, Morrisey et al. 2016). All other commercial diving operations contacted in New Zealand had limited or no experience of vessel wrapping.

The wrapping procedure usually involves divers deploying a single sheet or multiple sheets of plastic which are then joined along the seams with tape by divers to complete an enclosure. The volume of enclosed water can be reduced by pumping out, and biocides can be added, with chlorine, acetic acid, or naturally developing anoxia being used to treat the biofouling. The service provider now uses granulated chlorine as its preferred biocide and holds its own consent for use and discharge of the neutralised byproducts only in the Nelson region. While they follow the recommended protocol for chlorine use (Morrisey 2015), they prefer to extend the treatment period for as long as possible to eliminate more resilient biofoulers, especially bivalves which have been observed to be particularly resistant to treatment with chlorine.

The company has treated around 70 vessels in this manner over the last 8 years, including a naval frigate of 115 m in hull length. The wrapping method can accommodate a wider range of vessels, than other enclosure methods, as it sufficiently flexible to deal with unusually shaped vessels, such

as barges and dredges, which often can be more difficult to haul out or may not have suitable haul out facilities available nearby. For these vessels particularly, the wrapping technology is reportedly more cost effective and hence it has been more extensively used than those enclosure systems that can treat biofouling on smaller and more easily handled vessels. Wrapping enclosure requires certified commercial divers with experience in implementing the procedure. Also, the vessel needs to be moored in suitable conditions with low water and wave movement to facilitate the wrapping procedure and to maintain the integrity of the enclosure once fitted in place. Additionally, the experience of the dive operators in applying the wrapping is reportedly critical to ensuring the enclosures are well sealed.

Overall, in-water enclosure and encapsulation methods for the treatment of marine biofouling on vessel hulls, whilst relatively new in New Zealand, are well established as effective tools for marine biosecurity interventions. These methods complement the traditional approach to intervention of using vessel haul out and hull cleaning. The limited use of in-water treatment systems is largely due to their inconvenience and cost of deployment. Where they are used, chlorine is almost always applied as a biocide, which is reportedly highly effective against soft-bodied biofouling organisms which are of greatest current concern in New Zealand. However, this treatment appears to be not fully effective against biofouling organisms with hard exoskeletons, for which a number of potential invasive species pose a significant risk to New Zealand waters.

It is also likely that biofouling in hard to reach locations, such as sea chests and pipework, may also provide refuges from biocidal treatment in enclosure systems (Growcott et al. 2016, Cahill et al. 2018). The extent to which the enclosure treatments are ineffective for removing all biofouling from vessel hulls in a variety of situations has not been documented, and it would appear that there is no systematic survey and record keeping of residual biofouling following the treatment of hulls when using enclosure systems on a routine basis. Given the diversity of taxa that can contribute to biofouling on a vessel hull, as well as the variability in the extent of the biofouling, the diversity of hull types, and treatment conditions (e.g., water quality of enclosed seawater), it is highly likely that effectiveness of enclosure treatments will vary in response to some of these variables. Those entities implementing enclosure treatments appear somewhat uncertain of the level of effectiveness of enclosure treatments, and all of those individuals interviewed in the course of this review, referred to erring on the side of caution by extending recommended treatment times and doses as a practical means for addressing this uncertainty. Ensuring structured monitoring of treatment events using enclosure systems in future would help to improve the available knowledge of this approach to managing the risk of introduction of marine NIS via hull biofouling. Likewise, the availability of the existing enclosure systems provide an excellent opportunity to undertake more structured research toward verifying the effectiveness of biocide application protocols.

**BIOCIDES and EFFECTIVENESS**

1. **Biocides**

Encapsulation systems have been successful against target biofouling species where the enclosed environment has simply become anoxic (Coutts and Forrest 2007, Inglis et al. 2012, Atalah et al. 2016). However, the addition of biocidal chemicals has been shown to accelerate the process of

killing target species fouling the hull of vessels. An extensive list of potential biocides for in-water systems and vessel sea chests has previously been compiled for treating in-water systems to remove or treat biofouling in vessel sea chests and internal pipework (Growcott et al. 2016). In comparison, the biocides for hull encapsulation and enclosure treatments will be applied to larger and more exposed surfaces that are likely to support a larger biomass of attached biofouling. Nevertheless, the selection of biocides in all the systems mentioned need to meet the following criteria:

a) **Effectiveness** against a broad scale of biofouling taxa in their juvenile and adult stages. Ensuring mortality of a wide range of biofouling taxa, including resistant species, is of fundamental importance in the selection of a biocide.

b) **Compatibility** with different materials of vessel hulls and the encapsulation/enclosure material. The biocide treatment should not cause corrosion or damage of the hull, or deactivate any antifoul coatings, or damage the enclosure material, especially if it causes it to leak. This concern is discussed by Morrisey and Woods (2015) who conclude that the reason for the treatment needs to be initially determined, as it could be that there are only isolated patches of biofouling to treat on hulls, which otherwise have hull antifouling coating that is working effectively.

c) **Feasibility** relates to the cost and ease of use of the biocide. A feasible biocide will be economical in terms of the costs of purchase and use. The biocide needs to be commercially available in sufficient quantities. The biocide should also exhibit potency at realistically applicable concentrations for practical exposure times, such as exposure periods of less than 48 hours (Aquenal 2009, Inglis et al. 2012). The durability of the biocide also needs to be considered because biocide activity against biofouling and biodegradation processes will decrease the effective concentration of the biocide in the enclosed water, potentially necessitating the addition of further biocide to maintain the toxic dose.

d) **Compliance** relates to extent of the regulatory controls over the use of a particular biocide. The use of the biocide in enclosure systems should comply with a mix of government regulations covering the risks to personnel applying the biocide, as well as people and property within the vicinity of the biocide, and the potential environmental impacts associated with the use of the biocide. Consequently, the relevant regulatory regime includes the Resource Management Act and the planning and policy documents it gives rise to, the Hazardous Substances and New Organisms Act and associated regulations, and the Health and Safety at Work Act which manages the risk to personnel from the handling and use of biocides.

e) **Disposability** relates to the ease of the disposal of waste biocide or its breakdown products. Encapsulation and enclosure treatment systems are advantageous in that the biocide treatments and their breakdown products are retained within the enclosure, and this can allow for them to biodegrade or be neutralised before the release of encapsulated water prior to release to the wider marine environment. The alternative of recovering the encapsulated water for treatment and disposal elsewhere is likely to be impractical in most situations where enclosure systems are deployed at sea.

**1.1 Biocidal mode of action.**

The mechanism of action of the biocide plays a major role in determining its effectiveness against a range of marine biofouling species, with a strong preference for biocides that are effective against as many species as possible. Established marine biofouling is typically highly taxonomically diverse and can include representatives from 15 or more different phyla, including both prokaryotes and eukaryotes, with species ranging from colonies of cyanobacteria, through macroalgae, and chordates. Such an immense diversity of organisms has a correspondingly enormous mix of morphologies, cellular and bodily construction, biochemical composition, metabolism, and behaviours. Consequently, a biocide must possess broad activity against a diverse range of organisms in order provide a high degree of effectiveness against biofouling. For example, some biofouling species can tolerate some biocides through innate resistance mechanisms that prevent the biocide reaching its site of action, such as the detection and closure of shells in mussels (Martin et al. 1993). This aspect is important because a number of the species of concern as potential invasive marine NIS are bivalves, such as the Asian clam (*Potamocorbula amurensis*), or are secondary target species that are present in localised locations around New Zealand, such as the Asian bag mussel (*Arcuatula senhousia*).

The principle mechanisms of biocide action have been reviewed and defined by Tsolaki and Diamadopoulos (2010) as:

a) Damage to the cell wall,
b) Alteration of cell permeability,
c) Alteration of the colloidal nature of the cell protoplasm,
d) Alteration of DNA or RNA structure, and/or
e) Inhibition of enzyme activity.

Biocides are further classified by their chemical reactivity as oxidizing or non-oxidizing (Tsolaki and Diamadopoulos 2010).

Oxidising chemicals have a broad spectrum of activity, with a generally accepted mode of action that involves the oxidation of chemical bonds in important organic molecules including proteins and lipids. As such these compounds have a number of targets and their lethal action is from a loss of cell integrity through generating damage to cell walls and cell membranes which eventually leads to the loss of cellular structure. Therefore, oxidising biocides are more likely to provide the broad range of activity required for an in-water treatment. They are also likely to be active against surface structures, such as calcareous exoskeletons, that would otherwise provide the target species with an innate protection against harmful chemicals. The disadvantage of a mode of action that exhibits a broad range of target organisms is that off-target effects can dilute the effective concentration. For example, biocides that react with a broad range of organic materials will be rapidly depleted when applied in situations where there is a high level of organic material, as would be expected in seawater in many coastal locations, or where there is a high level of biofouling. Additionally, off-target oxidation may cause damage to other materials, such as the encapsulation materials and the vessel hull and any associated paint or coating. Therefore, the consideration and possible mitigation of these disadvantages will need to be required when choosing a biocide for in-water application within an enclosure. Oxidising biocides include chlorine, chlorine dioxide, ozone, bromine, hydrogen peroxide and peroxyacetic acid.

Non-oxidising chemicals cover a broader range of chemicals and modes of action. In general they will have a more targeted mode of action and can act by interfering with the metabolism of

organisms, rather than just reacting with important molecules. The non-oxidising biocides are typically more specifically effective against certain taxa, and so frequently suffer from a narrower spectrum of activity, or are subject to high levels of innate tolerance, or are prone to the development of resistance. In general, the non-oxidising biocides are normally also effective at higher dosages, which can increase costs and raise issues around feasibility of both application and disposal procedures. However, the narrower range of targets means that they are much less likely to be corrosive to various materials potentially increasing their usefulness for in-water enclosure application (Grandison et al. 2011). Examples of non-oxidising biocides include acetic acid, formaldehyde, glutaraldehyde, organosulphur compounds, quaternary ammonium salts, and anionic and non-anionic surfactants.

## 1.1 Biocides with potential for encapsulation

Oxidising and non-oxidising biocides with potential for in-water treatment of biofouling have been extensively reported (Chattopadhyay et al. 2004, Anderson 2005, Coutts and Forrest 2007, Piola et al. 2009a, Grandison et al. 2011, Morrisey and Woods 2015, Roche et al. 2015, Atalah et al. 2016, Growcott et al. 2017, Cahill et al. 2018). However, this anticipated potential is predominantly based on the extrapolation from studies of other biocidal applications, for example on-ship treatments of ballast water, and/or laboratory-based studies of single organism toxicity, which are prone to experimental artefacts (Martin et al. 1993). Investigations providing evidence to inform the treatment of complex biofouling communities with standardised in situ reagents for encapsulation systems is lacking (Atalah et al. 2016). Of the studies undertaken to date, some have focused on the hypoxic effect of encapsulation to kill the fouling organisms (Mantelatto et al. 2015, Atalah et al. 2016), while others have investigated the addition of freshwater or chemicals such as acetic acid or chlorine to accelerate the killing of biofouling (Anderson 2005, Coutts and Forrest 2007, Roche et al. 2015, Atalah et al. 2016).

The following sections provide an overview of the information available on in-water chemical treatments used to target marine biofouling. Other treatments that have proven effective in ballast water and waste water treatment are also reviewed, as they may have further potential for the enclosure treatment systems. The analysis of biocides is structured to consider the key criteria for as outlined above, i.e., Effectiveness, Compatibility, Feasibility, Compliance and Disposability in turn. The biocides considered include both oxidising biocides (including chlorine and other chlorine-based compounds, ferrate, hydrogen peroxide, iodine, ozone, peracetic acid, and the less powerful oxidisers sodium hydroxide and copper sulphite) and non-oxidising agents (including acetic acid, quaternary ammonium compounds (QACs), bromide, calcium hydroxide, descaler formulations, hydrogen sulphide, and sodium metasilicate). However, only acetic acid and chlorine currently have sufficient test results to suggest their use in encapsulation systems when also considering environmental risk and regulatory compliance (Coutts and Forrest 2005, Roche et al. 2015, Atalah et al. 2016).

### 1.2.1: Biocides with previous success

#### Acetic acid

Acetic acid consists of a methyl group attached to a carboxyl group that is classified as a weak acid. It is a colourless liquid organic compound. Glacial acetic acid contains a very low amount of water (less than 1%) and concentrations of acetic acid will be given here as a percentage by volume, with glacial acetic acid being 99%, and standard household vinegar being in the range of 3-9 %.

Various studies give evidence to support the biocidal activity of acetic acid against biofouling species, although the concentration and contact time required for particular target species can vary.

Laboratory controlled immersion in 4-5% acetic acid is a fast (<24 hr) and effective means of complete eradication for a variety of marine biofoulers including the tunicates *Ciona intestinalis* (Forrest et al. 2007), *Styela clava* (Coutts and Forrest 2005), and *Didemnum vexillum* (Piola and Conwell 2010, McCann et al. 2013), as well as terebellid polychaetes (Forrest et al. 2007).

For a range of cosmopolitan fouling taxa, a 4% solution kills many soft-bodied organisms after 1 minute (Forrest et al. 2007). However, this approach was designed to manage biofouling in mussel farms, where the primary research aim was to identify antifouling concentrations with minimal effect to mussels (Forrest et al. 2007). Acetic acid treatments at higher concentrations and/or longer contact times can overcome defences of more resistant taxa such as mussels and bryozoans by degrading calcareous exoskeletons. For example, a 5% solution of acetic acid applied for a period of 48 hours was reported to be 100% effective for the removal of mussels and bryozoans (Atalah et al. 2016). Soft bodied biofouling species appear to be more vulnerable to exposure to acetic acid. For example, a single spray application of 10% acetic acid over 30 minutes removed up to 95% of *D. vexillum* cover, with increased success with repeat applications (Piola et al. 2009a). The same study showed *D. vexillum* being eradicated to 99% with repeat-spraying at exposure times of 1 minute (Piola et al. 2009a). Likewise, exposure to a 5% solution of acetic acid for 30 seconds resulted in 95% mortality of the common fouling tunicate, *Ciona intestinalis* (Carver et al. 2003). Preliminary immersion experiments demonstrated that full mortality of *Styela clava* was obtained with a 1 minute immersion in 5% acetic acid and a 10 minute immersion in 1% acetic acid (Coutts and Forrest 2005).

The application of 1% acetic acid treatments to pontoons placed into enclosures, where *Styela clava* had been transplanted alongside a range of established native and introduced biofouling taxa, consistently obtained 100% mortality of the *S. clava* after 10 minutes exposure to the acetic acid solution (Coutts and Forrest 2005). Most other taxa succumbed to the 1% acetic acid exposure, which extended up to 12 hours of exposure. However, Pacific oysters (*Crassostrea gigas*) and the calcareous tubeworm (*Pomatoceros terraenovae*) both survived even 12 hours of exposure, although the concentration of acetic acid had fallen to levels between <0.1 and 0.6% by the end of the exposure period.

Other studies have shown the effectiveness of acetic acid against a range of taxa. Small scale field experiments found a 100% efficacy against the Pacific oyster, *Crassostrea gigas*, after exposure to 5% acetic acid (from household vinegar) for both 5 and 10 minutes (Rolheiser et al. 2012). Mortalities for threespine stickleback and sand shrimp were reached with 320 mg L$^{-1}$ (approximately 0.032%) and 500 mg L$^{-1}$ (approximately 0.05%) acetic acid, respectively, after an exposure of 96 h (i.e., 4 days) (Locke et al. 2009).

Acetic acid has been used as a biocide for recent studies of encapsulation and enclosure technologies for vessel hulls. Four-year-old and three-month-old communities of biofouling displayed a reduction in cover to around 2.5 ± 25% and 8 ± 4%, respectively, after one hour of small scale enclosure with 5% acetic acid (Atalah et al. 2016). Total mortality of the fouling communities was achieved within 24 hours for the four-year-old community and within 48 hours for the three-month-old community. The excellent performance of acetic acid here extended to overcoming the defences of taxa able to survive encapsulation without the addition of biocide. Mussels encapsulated with acetic acid were observed to have weakened and crumbling valves, in support of

Forrest et al. (2007), who indicated that acetic acid is effective against more resistant taxa, such as mussels and bryozoans, because it can dissolve their calcareous exoskeletons.

Compatibility with hull and encapsulation material:

Acetic acid at a concentration of 4% or 5% has been applied in situ on a number of occasions in New Zealand at small scale and on small to intermediate size vessels, particularly in encapsulation trials using polyethylene silage wraps (Piola et al. 2009b, Roche et al. 2015, Atalah et al. 2016). It has also been tested separately on 27 different vessels of between 7 to 30 m in hull length with 100% efficacy over 7 days (Pannell and Coutts 2007). This experience can be interpreted as evidence suggesting that acetic acid at concentrations up to 5% will not cause any compatibility issues as it could reasonably be expected that they would have been identified during these applications. Acetic acid has been implemented as a biocide on at least one large scale project, on a vessel exceeding 100 m with limited success, which was possibly due to structural failure of the encapsulating wrap caused by unsuitable water current conditions at the location of deployment of encapsulation (Golder Associates 2008, Bruce Lines, Diving Services New Zealand Ltd, pers. comm.).

Feasibility: Economic viability, commercial availability, and regulatory compliance:

Acetic acid is readily commercially available, and is relatively inexpensive. Health and safety considerations do present a barrier to the use of acetic acid because when treating a large vessel substantial quantities of concentrated acetic acid may be required to achieve a 5% acetic acid solution in the encapsulated water. Concentrated acetic acid as a 99% solution is often termed glacial acetic acid because it solidifies into acetic acid "ice" at temperatures below 16.7 $^{\circ}$C. The New Zealand EPA lists acetic acid as flammable with a flashpoint of 39 $^{\circ}$C (Classification 3.1C), hazardous and acutely toxic as liquid and vapour (6.1D), and describes it as "slightly harmful in the aquatic environment or [is] otherwise designed for biocidal action" (9.1D) (https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/view/609).

Disposability:
The dominant environmental fate process for acetic acid in water is ready biodegradation under aerobic and anaerobic conditions. Neutralisation via addition of a strong base (e.g., sodium hydroxide), before releasing the encapsulation water into the environment is also possible (Yu et al. 2000). The neutralisation reaction ($CH_3COOH + NaOH$ (s.b) $\rightarrow CH_3COONa + H_2O$) produces sodium acetate, which is listed as a chemical of low concern with an oral dose toxicity of $LD_{50}$ (lethal dose for 50% of individuals) for rats between 3,500 and 25,956 mg kg$^{-1}$ (pubchem.ncbi.nlm.nih.gov/compound/Sodium_acetate#section=Top). There has been little investigation into environmental effects of a sustained or an acute release of sodium acetate or acetic acid into the marine environment, however, some linkage has been made between continued pollution (including sodium acetate) selecting for growth of methanogenic archaea in estuarine sediment (Saia et al. 2010). Continued acetic acid and acetate disposal could potentially contribute to the acidification of estuarine and seawater in the local vicinity of the site of disposal in the event of low tidal flushing (Locke et al. 2009).

Summary:

Acetic acid has shown promising results on small scale for use in encapsulation treatment systems for biofouling, however when treating a large vessel a substantial volume of glacial acetic acid (99%)

may be required to achieve a 5% acetic acid solution in encapsulated water. For example, using the estimates of encapsulated volume of water provided in Morrisey (2015) for a yacht with a hull length of 8 m, (between 1.1 and 22.4 $m^3$ or between 1100 and 22,400 l of enclosed water) between 50 and 1120 litres of 99% acetic acid would be required to obtain a 5% acetic acid solution. It is likely that additional acetic acid would be required to maintain a level of 5% over any duration due to biodegradation. Due to the potentially dangerous nature of concentrated acetic acid, safer alternative biocides that are easier to handle would appear to provide a more favourable solution (Morrisey et al. 2015, Atalah et al. 2016).

Research Gaps:

There is good evidence that acetic acid is an effective biocide against a myriad of hull biofouling taxa. However, there remains a significant lack of operational guidelines for the use of biocides, including acetic acid, within encapsulation systems, as highlighted by Atalah et al. (2016). The efficacy of acetic acid for this specific application requires further quantification to determine optimum acid concentration to maximise mortality while conserving hull and environmental integrity. Therefore, it will be of importance to investigate the effects of acetic acid exposure on commonly-used anti fouling paints and galvanic surfaces for vessels that are suitable for encapsulation. Furthermore, reduction of the dose of acetic acid required to achieve efficacy, possibly by extension of exposure times, may also assist in reducing the risks associated with handling larger quantities of concentrated acid required to achieve higher effective doses.

### Chlorine

Chlorine has been used as a disinfectant for many years and has been extensively studied as a biocide with regards to its chemistry, toxicity, and ecotoxicity (Stewart et al. 2001, Rajagopal et al. 2012). Chlorine-based disinfectants and biocides come in a variety of different chemical forms which have different properties and costs. Commonly used forms of chlorine-based disinfectants include the hypochlorites, sodium hypochlorite (a liquid – usually the basis of household bleach), lithium hypochlorite and calcium hypochlorite (both solids). Hypochlorite is broken down by ultraviolet radiation, releasing the chlorine as gas into the atmosphere, and so is susceptible to inactivation by sunlight. In other commonly used forms of chlorine-based disinfectants the chlorine is stabilised by cyanuric acid, such as in dichloroisocyanurate dihydride (dichlor – as widely used in swimming pools) and trichloroisocyanuric acid (trichlor). Dichlor is the sodium salt of dichloroisosyanuric acid (sodium dichloroisosyanurate di-hydrate, $C_3Cl_2N3NaO_3.2H_2O$, CAS # 51,580-86-0), contains 56% chlorine by weight and reacts with water to produce hypochlorous acid, a powerful oxidiser, and cyanuric acid which has low acute toxicity.

The Free Available Chlorine (FAC) is the key measure of chlorine available in water, in the form of dissolved gas ($Cl_2$), hypochlorous acid (HOCl), or hypochlorite ion ($OCl^-$), to act as a disinfectant. The FAC decreases if hypochlorite is inactivated by sunlight, and when the chlorine reacts with organic material. The FAC can be easily monitored to ensure that the chlorine remains at an effective biocidal concentration by additional dosing of the source compound.

The generation of trihalomethanes (THMs) as disinfection by-products, following reaction between the chlorine used for disinfection and organic matter in the water is a potential disadvantage of using chlorine (Mayack et al. 1984). At elevated levels THMs have been associated with negative health effects such as cancer and adverse reproductive outcomes in mammals (Cortés and Marcos 2018). However, these health effects are associated with the disinfection of drinking water and swimming pool water, where safe levels are now regulated. THMs are unlikely to impact the use of chlorine disinfectants in enclosure applications, although it may be prudent to evaluate the potential for inhalation of these compounds further in the event of the selection of chlorine as a biocide for more extensive use in encapsulation treatments.

A less commonly used form of chlorine-based disinfectant is chlorine dioxide, which is a stronger biocide than chlorine and is effective over a broader pH range. In solution it does not react with ammonia, bromine or nitrogenous compounds and does not form trihalomethanes (Mayack et al. 1984). Instead, by-products generated by oxidizing reactions of chlorine dioxide in solution are sodium chlorite, chlorate, and chloride, which are generally considered acceptable for discharge by regulatory bodies and do not lead to corrosion (Rajagopal et al. 2012).

Effectiveness: Biocidal spectra and time frames:

Various studies provide evidence to support the biocidal activity of chlorine against marine biofouling species, although the concentrations used and contact times tested do vary, as do the particular target species. Hypochlorites, dichlor and trichlor are well established biocides that have been applied in several control operations for marine NIS and are generally found to be effective against various biofouling taxa (Mayack et al. 1984, Bax et al. 2002, Coutts and Forrest 2007, Rajagopal et al. 2012, Morrisey et al. 2016). Chlorine has several biocidal functions including direct toxic effects on adult organisms, the prevention of settlement and growth of larval stages, and the reduction of the attachment strength of biofouling to the substrate (Growcott et al. 2016).

Laboratory experiments with the colonial ascidian *D. vexillum* treated by immersion in 0.5 – 2% dilutions of sodium hypochlorite containing commercial bleach formulations for 20 seconds and up to 2 minutes gave 100% mortality (Denny 2008). Likewise, immersion of *D. vexillum* colonies in 1% sodium hypochlorite solution in seawater for 15 and 30 minutes resulted in 80% reduction in colony size after two weeks (Roche et al. 2015). The subsequent enclosure and treatment of a pontoon fouled with colonies of *D. vexillum* with sodium hypochlorite at 0.05% (500 mg $l^{-1}$) was effective in reducing the surface area of the fouling colony by 91% after 12 days.

Extending the application of chlorine to the treatment of *D. vexillum* on vessel hulls using an initial FAC at ~200 mg $l^{-1}$ over 12 hours resulted in 100% mortality of the colonial ascidian (Pannell and Coutts 2007). Dichlor delivering an initial FAC of 200 mg $l^{-1}$ in seawater was used to successfully treat fouling consisting mainly of the Mediterranean fanworm, *Sabella spallanzanii*, on the outer hull of an 8 metre long yacht held within an encapsulated dock for 16 hours (Morrisey et al. 2016). The concentration of FAC decreased within the floating dock from 200 to 50 mg $l^{-1}$ after only 2 hours

and to less than 10 mg l$^{-1}$ after 16 hours. A sample of 30 individual fanworms was collected from the hull to evaluate the success of the treatment and all showed morphological damage, with 28 having no response to the touch, which is generally considered a reliable indicator of mortality. Re-examination of the hull after 6 days indicated all target organisms were killed.

Piola et al. (2009a) evaluated sodium hypochlorite applied as a spray out-of-water in field trials using biofouling communities previously established on Perspex sheets that could be subsequently taken from the water for spraying. The fouling community was dominated by ascidians (*Ciona intestinalis*, *Botrylloides leachi*, *Botrylloides schlosseri*) and bryozoans (*Bugula neritina* and *Watersipora subtorquata*). Spraying the community with a 20% solution of a commercial bleach (5 g l$^{-1}$ of active chlorine) with a 12 hour exposure resulted in the removal of 75% to 100% of the biofouling based on observations of the surviving biofouling community 2 weeks following the spray treatment. However, closer observations at 2 weeks post-treatment highlighted that the spray consistently failed to remove the bryozoan *B. neritina*, although the other species were removed.

Laboratory experiments with 4 cm fragments of an invasive seaweed *Caulerpa taxifolia* achieved complete mortality at a concentration of 125 mg l$^{-1}$ of chlorine (supplied as sodium hypochlorite) following a 30 minute exposure (Williams and Schroeder 2004). A lower concentration of 50 mg l$^{-1}$ was not effective at destroying all the seaweed and was therefore not recommended for in situ eradication treatments. Further dilution of the chlorine to 10 mg l$^{-1}$ gave variable kill of the seaweed fragments, although it was not totally ineffective over an exposure period of between 30 and 120 minutes. Longer exposures were not tested, and although FAC measurements indicated between 75 and 90% of the original chlorine dose remained after a 120 minute exposure, it is clear from the results that for longer term treatment periods the effective concentration of chlorine would need to be maintained.

Experimental immersion of the solitary ascidian *S. clava* in seawater with a starting FAC of 50 mg l$^{-1}$ for 6 hours resulted in 100% mortality, although the level of FAC was found to deteriorate over time to only 2 mg l$^{-1}$ by the end of the exposure period (Coutts and Forrest 2007). However, chlorine was not as effective in the field trails of encapsulated marine pontoons, where transplanted *S. clava* and a range of the established taxa were not fully eradicated after 12 hours of treatment with a starting target concentration of chlorine of 200 mg l$^{-1}$ of chlorine. While 100% mortality of *S. clava* was observed in three experimental pontoons, in the fourth pontoon only 73% mortality occurred, mostly likely as a result of the greater amount of seaweed biomass that was present on this pontoon, which appeared to result in the FAC falling to < 1 mg l$^{-1}$ by the end of the 12 hour treatment. In the other enclosed pontoons where successful treatment occurred the 12 h FAC was ≥ 4 mg l$^{-1}$. Interestingly a number of biofouling taxa on the experimentally treated pontoons were observed to be impervious to the chlorine treatment including slipper limpets (*Crepidula costata*), oysters (*C. gigas*, *Saccostrea glomerata*), tubeworms (*Pomatoceros terraenovae*), sea squirts (*Asterocarpa cerea*, *Styela plicata*), macroalgae (*Ecklonia radiata*, *Codium fragile*).

Field studies of the effect of chlorine on green mussel, *Perna viridis,*, when delivered via sodium hypochlorite into the cooling waters of a power station, determined that a long term of exposure of 816 hours (34 days) to 1 mg l$^{-1}$ of chlorine at 29 °C was required to induce 100% mortality of the mussels (Rajagopal et al. 1995, Rajagopal et al. 2003). Exposure to a higher concentration of 10 mg

l$^{-1}$ resulted in 100 % mortality of mussels after 48 hours at the same water temperature. Masilamoni et al. (2002) also studied the effect of chlorine treatment, delivered as sodium hypochlorite, on *P. viridis* biofouling in the cooling waters of a power station. At a FAC of 9.1 mg l$^{-1}$ the induction of 100% mortality of mussels of 3–4 cm and 8–9 cm shell length size groups took 94 hours and 114 hours respectively. A lower concentration of 0.7 mg l$^{-1}$ FAC required 550-600 hours to achieve 100% mortality. The study highlighted the shell closure response of the mussel to FAC which provides the mussels with a high level of resistance to the biocide, with the results indicating that the response began at a chlorine level as low as 0.15 mg l$^{-1}$ and that complete valve closure occurred at 0.55 mg l$^{-1}$.

Investigations of another biofouling species of industrial water systems, the dark false mussel, *Mytilopsis leucophaeata*, demonstrated that lower concentrations of chlorine took much longer to eradicate the target species and only reached 100% mortality using 0.25 mg l$^{-1}$ after 89 days, which is again probably due to the ability of these bivalves to resist exposure to the biocide by shell closure (Rajagopal et al. 1997, 2002). Observations of the shell valve movements in this mussel species showed increasing shell valve closure in direct response to increasing chlorine concentration, with shell opening rates in controls without chlorine being ten times higher than occurred at 1 mg l$^{-1}$ (Rajagopal et al. 1997). However, responses by bivalves to chlorine are not consistent (Thompson and Richardson 1993, Rajagopal et al. 1997). For example, the common cockle, *Cerastoderma edule*, showed shell valve gaping and foot protrusion when exposed to a solution of 1.5–2.5 mg l$^{-1}$ sodium hypochlorite, and 100% mortality at concentrations above 2.5 mg l$^{-1}$ (Thompson and Richardson 1993).

Regardless, extended exposure times of greater than 48 hours that may be required to eliminate more resilient biofouling species are likely to be impractical in an enclosure treatment situation. Efficacy of chlorine for longer term exposures can also be affected by temperature, pH and co-occurring organic matter (Growcott et al. 2017).

The success of chlorine treatments against more resilient bivalves could be increased via the addition of a small quantity of chlorine-resistant surfactant (e.g., Dowfax 2A1, DOW Chemical Company), or with the addition of copper sulphate (Bax et al. 2002, Morrisey et al. 2016). In an attempt to eradicate the black-striped mussel, *Mytilopsis sallei*, from three marinas in northern Australia, initial laboratory tests indicated that >6 mg l$^{-1}$ of chlorine would be required and that 12 mg l$^{-1}$, added as calcium hypochlorite, would achieve 100% kill of the mussels in 111 hours (Bax et al. 2002). In the eradication attempts the water in the entire marina (600,000,000 l for the largest, Cullen Bay Marina) was adjusted to about 10 mg l$^{-1}$ of active chlorine (delivered as sodium hypochlorite), as the maximum achievable concentration. A high level of control in the invasive mussel was achieved in the Cullen Bay Marina in this manner, substantially reducing the density of the mussels during a 6 day exposure, but ultimately failed to completely eradicate the mussel (Bax et al. 2002). The subsequent successful eradication of the mussel species from this marina was only achieved with a treatment combining copper sulphate with the sodium hypochlorite, after a successful trial in the smaller Tipperary Waters Estate Marina (Bax et al. 2002). Conjunctional use of copper sulphate with a base chlorine treatment has also been successful in further targeting other resistant taxa such as polychaete worms (Morrisey et al. 2016). However, despite the success of this

approach, the toxicity of copper released into the marine environment is of particular concern that will be discussed further in a later section of this report.

Trials of chlorine dioxide have been reviewed by Growcott et al. (2016), with two studies that show either 50% mortality of the zebra mussel, *Dreissena polymorpha*, at an exposure of 40 mg $l^{-1}$ for 3 to 6 minutes (Matisoff et al. 1996) and 100% mortality at 5 mg $l^{-1}$ after 70 minutes (Holt and Ryan 1997). Chlorine dioxide was also shown to be effective against early life stages of some biofoulers at 25 to 250 mg $l^{-1}$ (Hose et al. 1989). For a variety of biofouling, a concentration of 0.5 mg $l^{-1}$ for 9 hours applied continuously prevented further settling of biofouling such as barnacles, hydroids and serpulid worms (Jenner et al. 1998).

Compatibility with hull and encapsulation material:

Chlorine has been successfully tested on a variety of vessel hulls (Bax et al. 2002, Morrisey et al. 2016) inferring good compatibility with a range of hull types at 10 mg $l^{-1}$ over an extended timeframe. Chlorine is also widely used as an antibacterial agent on a number of other types of hard surfaces without issues being reported.

Feasibility: Economic viability, commercial availability, and regulatory compliance.

Chlorine is readily available commercially in several chemical forms and is relatively low cost. Liquid sodium hypochlorite and powdered calcium hypochlorite are available in large quantities, and would be preferred over the more expensive lithium hypochlorite. The physical form of chlorine as calcium hypochlorite as a solid pellet provides higher feasibility for use over liquid forms as this is easier to handle and administer.

Sodium hypochlorite reportedly costs around NZ $52 per 20 l (Aquenal 2009). Dichlor and trichlor are readily commercially available, with 3.6 kg of granular dichlor costing ~NZ$35 (Morrisey et al. 2016). Chlorine dioxide is around three times more expensive (Grandison et al. 2011) and is commercially available (Tsolaki and Diamadopoulos 2010).

Sodium hypochlorite is reported to be a 'green bio-dispersant' characterized by high biodegradability, an absence of bioaccumulation, low toxicity and non-carcinogenicity for human exposure (Coutts and Forrest 2005, Di Pippo et al. 2017). Dichlor satisfies all safety considerations as approved by the Environmental Protection Authority (2016) provided it is used in an approved manner for the treatment of biofouling in enclosure systems. Chlorine dioxide does have more significant safety issues that may limit its use in treating biofouling in encapsulation applications.

Disposability

Chlorine in solution can be readily neutralised prior to water discharge using thiosulphate (Morrisey and Woods 2015). Environmental considerations in studies where this approach has been used has not noted concerns for the release of the resulting reaction products in well circulated coastal areas (Morrisey 2015, Morrisey and Woods 2015), although in these studies major concern was the breakdown of residual chlorine. A stoichiometric approach to neutralisation of residual chlorine is

highly recommended, to ensure no excess thiosulphate addition and to best manage products and residuals in any water expelled into the surrounding environment.

The neutralisation reaction for chlorine in solution using thiosulphate is:

$$4\ NaClO + Na_2S_2O_3 + 2\ NaOH \rightarrow 4\ NaCl + 2\ Na_2SO_4 + H_2O$$
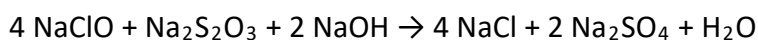
Which forms products that are considered of negligible environmental or toxic risk when released into the marine environment (https://www.heraproject.com/RiskAssessment.cfm?SUBID=39).

The ideal conditions for the dissipation of released residuals following the use of this biocide in an encapsulation treatment are contrary to those preferred for establishment of an encapsulation treatment around a vessel, i.e., low natural water movement around the hull. However, sufficient dilution and tidal movement, even within a relatively enclosed marina, should be sufficient for the rapid dilution and dispersion of the discharge from an encapsulation treatment.

Summary

The available evidence suggests that chlorine is effective against many biofouling species of concern when used in an enclosure or encapsulation situation. Although much of the research has been performed with liquid sodium hypochlorite, the handling and administration of solid sources of chlorine, such as calcium hypochlorite and dichlor, is better suited for use in enclosure treatments.

The instances of failure of chlorine treatments in killing biofouling have been linked to the rapid deterioration of FAC levels leading to reduced efficacy, and resistance by some organisms, especially those with hard exoskeletons and closure behaviour. The rapid deterioration of FAC appears to be most pronounced in situations with high levels of organic biomass, and therefore to maintain the efficacy of chlorine as a biocide it is essential that FAC is continually monitored over the treatment periods and additional dosing should occur when FAC falls below the target concentration. This will also help to avoid excessive use of the chlorine product. In the event of some biofouling taxa exhibiting resistance to chlorine there is the opportunity to combine additional chemical treatments with chlorine to improve the overall efficacy. For example, copper sulphate has been successfully used in conjunction with chlorine for this purpose for elimination of more resilient marine taxa (Bax et al. 2002), however, copper is highly toxic to many marine organisms (see later section dealing with copper sulphate as a biocide, Pimentel 1971, U.S. Environmental Protection Agency 2009 - toxnet.nlm.nih.gov). Surfactants, or biological relaxants, such as magnesium chloride, may also provide alternative chemical combinational treatments with chlorine that could provide higher overall biocidal efficacy for more resilient biofouling species.

Research Gaps:

While the effectiveness of chlorine treatment on various biofouling species has been quite extensively reported, there is an overall lack of studies which have reported on well controlled in-

field enclosure treatments of biofouling on vessel hulls. This includes reporting on the effectiveness of treatment against a variety of biofouling species, especially those more likely to be resistant to chlorine as a biocide.

The presence of a surfactant or muscle relaxant should increase the bioavailability of any chlorine taken in by bivalves (e.g., for intermittently opening or gulping bivalves), resulting in a more rapid biocidal effect. However, this would need to be validated with further testing.


### 1.2.2: Other biocides with potential:

**Quaternary Ammonium Compounds**

Quaternary ammonium compounds (QACs) contain at least one hydrophobic hydrocarbon chain linked to a positively charged nitrogen atom, and other alkyl groups which are mostly short-chain substituents such as methyl or benzyl groups. QACs are used commercially as non-oxidising surface disinfectants and are widely used in household and industrial products (Martín-Rodríguez et al. 2015). They are also used in industrial water cooling systems as a biocide (Zhang et al. 2015). Laboratory and field use of QACs to control biofouling have been made with a variety of commercial formulations, making comparisons difficult between different treatments using different formulations.


Effectiveness: Biocidal spectra and time frames

Formulations including QACs have been found as effective molluscicides (i.e., controlling molluscs) and include benzalkonium chloride and didecyl dimethyl ammonium chloride. Commercial formulations are applied as 1-5% detergent solution for 24 hours against mussel fouling in Australian Navy vessels (Piola and Grandison 2013). Treatment solutions were effective at killing large sized mussels (*Mytilus galloprovincialis planulatus*) in pipework and sea chests following a 24 hour dosing period, but  small-sized mussels (0 – 30 mm) could only be killed with 5% solutions (Piola and Grandison, 2013). Laboratory tests on *Mytilus galloprovincialis planulatus*, further demonstrated the lethality of QACs at 1% dilutions over a 6 h exposure (Lewis and Dimas 2007). Neil and Stafford (2005), undertook laboratory tests to establish effectiveness against the rock oyster *Saccostrea glomerata* as a model bivalve species. The failure of 10 % solutions to kill the oyster indicated a narrow spectrum of lethality. Other studies have demonstrated the efficacy of QACs against a range of freshwater and saltwater mollusc taxa, one example being Britton and Dingman (2011), who showed that a 3% solution of a commercial QAC formulation resulted in 100% mortality of freshwater Quagga mussel (*Dreissena rostiformis begnsis*) veligers within 60 minutes.

It is important to note that some QAC studies gave comparisons with other biocides (Neil and Stafford 2005, Lewis and Dimas 2007). In these studies 4% or 6% acetic acid was more effective.

Compatibility with hull and encapsulation material.

Britton and Dingman (2011) indicate correct usage inflicts minimal damage on currently recognised treatment areas, which are predominantly internal pipework and sea chests.

Feasibility: Economic viability, commercial availability, and regulatory compliance.

As the studies on efficacy indicate, a number of QAC formulations are available, and in common use. QACs are reported to degrade under aerobic conditions, with reductions to 66% obtained in 29 days (Piola and Grandison 2013). When used according to label, QACs should not have toxic effects. However, benzalkonium chlorides are listed as hazardous, toxic and corrosive substances (https://www.epa.govt.nz/database-search/approved-hazardous-substances-with-controls/view/2434) and so appropriate care would be needed in protocols for use.

Disposability

Neil and Stafford (2005) determined approximately 8000 l of seawater would dilute 1 L of 5% QAC solution effluent sufficiently for safe release into the environment, which might imply applicability for molluscan infestations on a targeted basis where other biocides prove insufficient. External usage of the biocide within the encapsulation may prove similarly effective on molluscan infestations, but substantial amounts of seawater are required for sufficient dilution and so release may pose an ecological risk in confined areas of minimal water flow, where of course the encapsulation system is most likely to be established.

Garaventa et al. (2012) tested three commercial biocides with active ingredients including a QAC (Didecyldimethylammonium chloride), an isothiazolone formulation (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) and an organophosphorus formulation Tetrakis (hydroxymethyl) phosphonium sulphate. Each showed efficacy against an invasive fouling mussel species *Brachidontes pharaonis* important in the Mediterranean, where the study was conducted, but effective concentrations were above the Discharge Limit Concentration (DLC, a threshold set by Italian regulations) for each product.

Summary:

While feasibly applicable at guideline concentrations, the sparse research on the biocidal effect of QACs on macrofauna fails to indicate a broad spectrum application outside of a molluscan biocide. Current usage is as a targeted approach against specific molluscan infestation and transmission (Neil and Stafford, 2005, Britton and Dingman, 2011, Garaventa et al. 2012) and the literature suggests applications in the eradication of resistant molluscan infestations, especially as in-water treatments of internal pipeline structures and sea chests.

Research Gaps.

A number of QAC formulations exist, and it will be necessary to evaluate which offer the best activity against both a range of biofouling species, and against the species that are more resilient to e.g. chlorine or acetic acid treatments. The literature investigated suggests that no severe adverse reactions to hull materials would occur, but the listed corrosive nature of benzalkonium chloride,

and the presence of additional components in commercial formulations, would make specific tests important to verify the safety of any recommended formations against hull materials.

It will also be important to assess long term ecotoxicity of multiple QAC releases into a single quarantine environment over time to properly manage and prevent ecotoxicity events.

### Bromine:

Bromine-based biocides are considered to be similarly effective to chlorination methods for the microbiological control of cooling waters and disinfection in water treatment systems (Moore et al. 2001, see Carpenter and Nalepa 2005 for a review). Bromines offer effective control at higher pH conditions (Grandison et al. 2011), however, they are affected by acidic pH, total dissolved solids and organic matter as the hypobromite ion (an ineffective biocide) will replace hypobromous acid (an effective biocide) (Walker et al. 2009).

Bromine is often added to chlorine treatments, especially in mildly alkaline waters (Grandison et al. 2011), however, there is an almost complete absence of studies assessing the efficacy of bromine as a sole agent for biofouling treatment.

Effectiveness: Biocidal spectra and time frames.

Laboratory and field data for bromine compounds applied to biofouling species is very limited. A dosage of $0.1 - 10$ mg $l^{-1}$ has been described as effective against bacteria, zooplankton and invertebrates over exposures of minutes to hours in cooling water (Chattopadhyay et al. 2004). Toxicity values of 0.52 mg $l^{-1}$ to fish (freshwater) bluegill sunfish (*Lepomis macrochirus*) (24 hours), 0.31 mg $l^{-1}$ to the rainbow trout (*Oncorhynchus mykiss*) (24 hours), 1.5 mg $l^{-1}$ to the invertebrate water flea (*Daphnia magnamagna*) (24 h) and 1 mg $l^{-1}$ (48 h) (WHO 2018).

Compatibility with hull and encapsulation material.

No specific data is available.

Feasibility: Economic viability, commercial availability, regulatory compliance and disposal.

Bromine-containing formulations are commercially available, but their cost may limit their use as they are typically about twice the cost of chlorine (Growcott et al. 2017). Several toxic by-products are formed when bromine is added to seawater, which may be of concern when considering release following treatment, although these products may rapidly degrade, potentially limiting environmental concerns (Chattopadhyay et al. 2004, Tsolaki and Diamadopoulos, 2010, Grandison et al. 2011).

Summary:

There is limited data available to evaluate the effectiveness of bromine compounds against biofouling species, however, what literature is available suggests effectiveness on a par or below that of chlorine compounds. The uncertainty around comparative effectiveness, coupled with the greater cost suggest that bromine is unlikely to be a preferred selection for a biocide in enclosure applications.

### Calcium (hydr)oxide (lime)

Calcium hydroxide is known as hydrated lime, while calcium oxide is more basic and is known as quicklime. Hydrated lime is used extensively in wastewater treatment in combination with ferrous sulphate (Gupta et al. 2012) and has been identified as a possible option for the treatment of biofouling (Inglis et al. 2012).

Effectiveness: Biocidal spectra and time frames.

Hydrated lime has been applied as a biocide in aquaculture at concentrations of 1–4% for 1 and 5 min exposures and it had no detrimental effects on oyster survival, growth, or their overall condition (Rolheiser et al. 2012). Solutions of 5 – 10% of hydrated lime with treatment times between 20 seconds and 2 minutes were tested on *D. vexillum* with the 2 minute exposure to 10% hydrated lime achieving close to 100% mortality (Denny 2008). However, in pilot studies of biocides that could be used as sprays on biofouling communities, hydrated lime appeared the least effective amongst a variety of other biocides for removing fouling taxa, with the removal 75–100% of biota reported for applications at high concentrations (10– 20%) over more than 6 hours of exposure (Piola et al. 2009a). It also appears that hydrated lime is more effective on some fouling species than others, with solutions of 4% hydrated lime causing significant mortality to the ascidians *C. intestinalis* and *Molgula* sp., while having little effect on the green macroalgae *Codium fragile* ssp. *tomentosoides* (Carver et al. 2010, Rolheiser et al. 2012).

Compatibility with hull and encapsulation material.

Specific studies on compatibility of hydrated lime and quicklime with vessel infrastructure are not available and would need to be undertaken for confidence in their use in encapsulation treatments.

Feasibility: Economic viability, commercial availability, and regulatory compliance.

Hydrated lime is readily commercially available, relatively safe to handle, and both Aquenal (2009) and Inglis et al. (2012) identify that it is by far the cheapest of the range of biocides they examined.

Disposability

The disposal of hydrated lime into seawater is not thought to be of concern (Locke et al. 2009).

Summary:
The effective dosages of lime products are achievable, especially given the low cost of lime (Inglis et al. 2012). Lime is effective against *D. vexillum,* a recognised New Zealand invasive (Coutts and Forrest 2007), however, it appears to have a narrower biocidal spectrum compared with other biocides and so is unlikely to be a good selection for encapsulation treatments, and especially those aimed at a wider range of biofouling species.

## Copper sulphate

Copper ions, alone or in complexes, have been used for disinfection purposes for centuries and are commonly used as water purifiers, algaecides, fungicides, nematocides, molluscicides, anti-bacterial and anti-fouling agents (Borkow and Gabbay 2005). Copper is highly toxic to amphibians, molluscs, nematodes and flatworms and slightly toxic to crustaceans and zooplankton, but is generally considered safe to humans (Pimentel 1971, U.S. Environmental Protection Agency, 2009, https://toxnet.nlm.nih.gov/). Copper oxides readily adsorb onto particulate matter and accumulate in marine sediment (Thomas and Brooks 2010). The aquatic fate of copper is highly dependent on the pH, concentration of organic matter, iron and manganese oxide and the hardness of water (Chattopadhyay et al. 2004). Copper sulphate is a commonly used form of copper as a biocide and is considered as a suitable biocide for encapsulation systems (Aquenal 2009, Inglis et al. 2012).

Effectiveness: Biocidal spectra and time frames.

In the eradication of invasive mussel species *Mytilopsis sallei* from marinas in Darwin, Australia, copper sulphate was used at a concentration of 4 mg $l^{-1}$ in combination with treatments of hypochlorite (Ferguson 1999, Bax et al. 2002). The accounts of the event suggest that copper sulphate was more potent than hypochlorite against the invasive mussel. Around 6,000 tonnes of copper sulphate were added to three marinas over two weeks, along with several tonnes of hypochlorite. The chemical achieved a high kill-rate within the environment and general toxicity levels subsided rapidly after treatments were concluded. There were no lasting environmental effects reported, or damage through corrosion to aluminium hulls of vessels in the marinas, although further knowledge needs to be gained given the toxicity of copper to a wide range of marine taxa (Ferguson 1999).

Compatibility with hull and encapsulation material.

No damage to aluminium hulls of vessels was observed within the Darwin marinas treated with copper sulphate, however, it appears as if this concern was not closely examined (Ferguson 1999). Effects of copper on new antifouling paints produced after the global ban of tributyltin (TBT) in the early 2000's must also be investigated also to assure viability, however, copper sulphate has

successfully been used recently in situ with no negative effects on hull materials being reported (Morrisey et al. 2016).

Feasibility: Economic viability, commercial availability, regulatory compliance and disposal.

Copper salts are commercially available, and easily obtained. Around 6,000 tonnes of copper sulphate were added to the three Darwin marinas over a period of two weeks, along with several tonnes of hypochlorite. The chemicals eliminated the target invasive mussel within the marinas and general toxicity levels subsided rapidly after treatments were concluded, with no environmental impact noted (Ferguson 1999). Copper sulphate is relatively safe to transport and handle, but is not currently approved for release into the marine environment in New Zealand.

Summary:

In situ experimentation has already been conducted in New Zealand with copper sulphate and determined that it is effective alongside other biocides as a narrow spectrum biocide for certain resistant taxa (Morrisey et al. 2016). While the biocidal activity may be of too narrow a spectrum for wide scale application, it might be particularly effective as a recommended additive to target and quickly eliminate biofouling species that are more resilient to other biocides, e.g. chlorine.

Research Gaps

The documented toxicity of copper sulphate is likely to raise objections, especially in communities reliant upon shellfish gathering. Research to determine how much copper sulphate would be needed for enclosure treatments of resilient species would help evaluate the risks. Research to provide an evidence based approach to the decision to use copper sulphate and the best regimen for application in a combined approach with chlorine, for example, would allow the controlled use of copper sulphate to restrict usage to effective levels only when necessary. It may also be possible to recover residual copper sulphate from enclosure water following treatment so that it does not need to be released to the environment, by passing it through an effective chemical adsorbent such as zeolite (Cabera et al. 2005).

### Descalers

Descalers are chemical formulations used to remove carbonates from cooling systems and are active by way of an acidic compound such as hydrochloric, phosphoric, sulfamic-citric or lactic acid. The acid reacts with carbonate compounds producing carbon dioxide and soluble salts and can degrade the calcium carbonate shells or casings of polychaetes and other carbonate forming organisms among biofouling organisms (Growcott et al. 2016). The key advantage of descalers over other chemical solutions, such as oxidising and non-oxidising biocides, is their capacity to chemically degrade and kill resilient taxa with calcareous exoskeletons.

<u>Effectiveness: Biocidal spectra and time frames.</u>

Several commercially available descalers of different chemical composition, based on hydrochloric acid, phosphoric acid or acid-surfactant combinations, were tested in laboratory experiments against mussels, *Mytilus* spp. (Bracken et al. 2016). The hydrochloric acid-based descalers were most effective, and achieved up to 100% mortality against after 8 hours of exposure.

The most commonly described commercial descalers in the literature are hydrochloric acid-based. Diluted descalers (25 % solution) applied for 8 hours was found to result in a 70% reduction in the biomass of *Mytilus* spp*.* largely through the dissolution of their shells (Bracken et al. 2016). However, the concentration of descaler required to produce effective control of *Mytilus* spp. after 24 hours of exposure was reported to be too high to be practical for application (Lewis and Dimas 2007). Likewise, the concentration required to be effective against the oyster *Saccostrea glomerata* after a 12 hour exposure was also reported to be too high to be practical (Neil and Stafford 2005).

<u>Compatibility with hull and encapsulation material.</u>

Concerns regarding the toxicity and corrosiveness to vessel materials from descalers are pertinent and would require further investigation to be confident of their safe use against vessel hull infrastructure and their coatings.

<u>Feasibility: Economic viability, commercial availability, regulatory compliance and disposal.</u>

Lewis and Dimas (2007) do not recommend the use of descalers on heavily-fouled surfaces because of low efficiency and suggest costs would be considerably higher than other available biocides.

Dependent on the product, most descalers are stated as being non-toxic, non-hazardous and disposable into wastewater treatment systems. Additionally, some descalers can be neutralised after treatment before release into the environment. Common commercial descalers are safe to handle and are effective against a broad spectrum of biofouling organisms, however, the longer term environmental impact of its release into the marine environment would need further study.

<u>Summary:</u>

The principle of descalers is attractive, given the promise of both killing and removal of calcareous biofouling species which are frequently more resistant to biocide interventions. The poorer effectiveness, low potency against key species and uncertainty around the longer term effects on environment and hull materials do not favour their selection as a biocide for encapsulation treatments.

**Ozone**

Ozone, $O_3$ gas, is a powerful oxidant that has found application in the disinfection of water systems. Ozone readily oxidises organic material on contact making it a useful broad spectrum biocide. Ozone

has been suggested as an effective disinfectant of cooling and ballast water due to its high toxicity during treatment and resulting non-toxic discharge, as the breakdown product is oxygen (Ashraf et al. 2014). Ozone reacts differently in seawater compared to freshwater due to the presence of bromide ions in seawater (Jones et al. 2006). Ozone oxidizes the bromide ions to bromine (hypobromus acid and hypobromite ion) and then to bromate. If seawater is ozonized for more than 60 min, essentially all bromide ions are converted to bromate. Bromate also has some toxicity to marine animals with the $LC_{50}$ (concentration required to kill 50% of individuals) ranging from 30 mg $l^{-1}$ bromate for Pacific oyster, *C. gigas*, larvae to several hundred mg $l^{-1}$ for fish, shrimp, and clams (Crecelius 1979).

Effectiveness: Biocidal spectra and time frames.

Low concentrations (0.1-0.3 ppm) of ozone achieve full mortality against some bacterial species levels after 15-30 minutes, but it is significantly less effective against sessile bacteria (Viera et al. 1999). Ballast water tanks have been treated with ozone gas diffusion for 5 and 10 hours with the ozone introduced into seawater rapidly converting to bromide (Br–) and to bromine based disinfectants (HOBr/OBr–). The most effective resulting reductions in viability observed were for 99.99% of culturable bacteria, 99% for dinoflagellates and 96% for zooplankton. For organisms suspended in the water column, sheepshead minnows usually had greatest mortalities, shore crabs least, and mysid shrimps exhibited intermediate mortalities (Jones et al. 2006). The effectiveness of ozone against marine biofouling species on vessel hulls has not been tested.

Feasibility: Economic viability, commercial availability, regulatory compliance and disposal.

Ozone can be generated on site with a combination of electrical discharge and oxygen gas with the required equipment, and the gas can be easily injected and dissolved into water. Such equipment is frequently used in freshwater and seawater treatment systems and is readily available, but usually in fixed installations. Ozone gas is readily detected by humans by smell at low concentrations, but exposure produces headaches, burning eyes and irritation to the respiratory passages. Accumulation of the gas in confined spaces can also create hazards. Ozone and its breakdown products in seawater, if released into the marine environment, would have limited toxic effects depending on the concentration and the period over which they have been in reaction with seawater and associated organic material. The ozone and breakdown products could be recovered by degassing and passing over activated carbon.

Compatibility with hull and encapsulation material.

The corrosiveness of ozone gas on metals depends on its concentration levels. Studies on mild steel in simulated seawater show that ozone has a preservative effect (Liao et al. 2012). However, ozone in damp air has an important role in aluminium corrosion (Oesch and Faller 1997). Ozone in air has a degradative, oxidative action on polymeric materials such as the polyethylene used as encapsulation wraps (Kefeli et al. 1971). However, given that ozone degrades when placed in seawater it is unlikely that reports of reactivity of ozone in air would be consistent with possible

effects in seawater. Further research of the possible effects of ozone and its breakdown products in seawater on vessel hull infrastructure would be needed. Ozone and a system combining ozone with hypochlorite are reportedly commercially available in some parts of the world (Tsolaki and Diamadopoulos 2010).

Summary:

Grandison et al. (2011) concludes that ozone is less effective than chlorine due to its rapid breakdown that would prevent an effective dosing regimen. The reduced efficacy of ozone at alkaline pH (levels in excess of pH 8.5), the formation of bromate by-products, its corrosive potential and the high cost of installation and operation (particularly in comparison to chlorine) suggest that it would not be a good selection for use in encapsulation treatments. The efficacy of ozone against biofouling species and its safety for use against hull and encapsulation materials would need further study before it could be used confidently in encapsulation treatments.

## Peracetic acid

Peracetic acid is a weak acid that does not exist in a pure form, but is present in a mixture of acetic acid and hydrogen peroxide. The recommended dosage in industrial cooling water systems is 1–10 mg $l^{-1}$ with a 1–3 hour contact time to kill bacteria like *Legionella pneumophila* (Jenner et al. 1998). It is widely used in the food industry at higher concentrations of 100 to 500 mg $l^{-1}$ to disinfect surfaces.

Effectiveness: Biocidal spectra and time frames.

Peracetic acid is the main component in a common commercially available formulation, which also includes hydrogen peroxide and acetic acid. An initial 100 ppm concentration of this formulation was found to eliminate more than 90% of the biomass of suspended microorganisms and 100% of phytoplankton within 48 hours after treatment (De Lafontaine et al. 2009). The treatment resulted in 100% mortality in caged fish exposed to treated waters but was found to be totally ineffective against adult zebra mussels and some nematodes living in tank sediments. By comparison, application of another commercial formulation obtained 100% mortality on 4 hour old embryos of *M. leucophaeata* and *D. polymorpha* with a 5 mg $l^{-1}$ exposure of 15 minutes (Verween et al. 2009).

Compatibility with hull and encapsulation material.

The corrosiveness and unstable nature of peracetic acid is a potential concern (Grandison et al. 2011).

Feasibility: Economic viability, commercial availability, regulatory compliance and disposal.

Peracetic acid is manufactured in New Zealand and is commercially traded. No mutagenic by-products are formed from reactions with organic matter and its decomposition products (i.e., acetic acid, water and oxygen), are considered as environmentally acceptable (Kramer, 1997), however

any continued use and disposal into the marine environment would need to investigate the potential for possible ecotoxicological impacts.

Summary:
Peracetic acid has good activity as a disinfectant against bacteria, but its poor efficacy against some biofouling species and uncertainty around its effects on vessel hull materials and coatings, plus the lack of longer term studies on its possible ecotoxicity in the marine environment do not support its selection as a biocide for encapsulation applications at this time.

**Sodium hydroxide (caustic soda)**

Sodium hydroxide is a highly caustic base that decomposes proteins and is expected to have a broad spectrum lethal effect when used as a biocide. It has been used in the chemical control of undesirable species in on-ship waters (TenEyck 2009) and in the aquaculture industry for disinfection (Kerrison et al. 2016).

Effectiveness: Biocidal spectra and time frames.

Solutions of sodium hydroxide at a concentration of 6 % (by weight) for exposures of between 30 seconds and 2 minutes were used as an experimental treatment against the colonial ascidian *D. vexillum*, which resulted in 100% mortality (Denny 2008). Sodium hydroxide in solution was also used to treat the fouling bivalve *Limnoperna fortunei* and reached a $LC_{50}$ value after 96 hours at 88.5 mg $l^{-1}$ (Montresor et al. 2013). Sodium hydroxide has been found to be effective in reducing the number of live organisms, from different taxa, including crustaceans, rotifers and green algae in laboratory studies (TenEyck 2009).

Compatibility with hull and encapsulation material.

Sodium hydroxide is a corrosive base that may have negative effects on both hulls and the encapsulation materials at the concentrations required for biocidal activity.

Feasibility: Economic viability, commercial availability, and regulatory compliance.

The commercial and household usage of sodium hydroxide is common and numerous cleaning resources dictate concentrations of 0.5-2% volume should be used. Unfortunately, these concentrations are lower than those required for efficacy against biofouling species, and new approvals as would be required for biocidal use in encapsulation and discharge.

Disposability

The neutralisation of sodium hydroxide requires either large amounts of a weak acid such as acetic acid, or reaction with a stronger acid such as hydrochloric acid. Depending on the acid used for

neutralisation, various products may require further management such as with chlorine species related to reactions with hydrochloric acid.

Summary:

Sodium hydroxide has shown promising results against target species in small scale experiments. However, it is unclear whether this potentially corrosive biocide will react with various marine surfaces and antifouling paints at the concentrations necessary to replicate success against fouling organisms on a large scale. In addition, while neutralisation is chemically straightforward, large quantities of acid may be required and the neutralisation products dealt with. For these reasons it is unlikely to be a preferred option for a biocide in encapsulation applications.

### Freshwater (Osmotic Shock) or Salinity Adjustment

Reducing or increasing the salinity of seawater can induce osmotic shock in marine organisms that can lead to their death (Tsolaki and Diamadopoulos 2010). Osmotic shock can be easily achieved by encapsulation treatment via addition of freshwater to decrease salinity, or salt to increase salinity levels. Salinity changes can lead to stress-induced spawning, but this is not of concern as the encapsulation should prevent the escape of any viable gametes, and for many marine organisms the larval stages are more vulnerable to biocides so they should be destroyed by the osmotic stress (Inglis et al. 2012).

Effectiveness: Biocidal spectra and time frames.

Freshwater treatment has proven to be very effective against fouling species, especially in aquacultural applications (Dunphy et al. 2005). Freshwater treatment for 180 – 300 minutes resulted in >98% mortality of the polydorid polychaete, *Boccardia acus*, mortality without producing any obvious harmful effects on oysters on which they were living. The same outcome applied to the fanworm *S. spallanzanii,* when treated in an aquaculture setting with green-lipped mussels, *Perna canaliculus*. After being immersed in hypo-saline water for 120 minutes, a 100% mortality rate in the fanworm could be achieved with no loss of mussels (Jute and Dunphy 2016). Adding freshwater is possibly the most cost effective and most environmentally safe method for treating biofouling. However, treatment times may extend to days and weeks in order to eliminate biofouling species which are tolerant to salinity fluctuations, which might not be feasible in encapsulation applications. For example, Pacific oysters are highly tolerant to a broad range of salinities, reported to be capable of surviving for over two weeks at salinity of 5 ppt and to grow at high salinity sites experiencing up to 48 ppt (see review in Wiltshire 2007). Hull biofouling species, especially those raising biosecurity concerns, typically have wide ranging environmental tolerances, including salinity, given their survival on hull surfaces.

Mussels covered in the colonial ascidian *D. vexillum* were dipped in freshwater for three periods (2, 5, and 10 minutes) and mortality of the ascidian was found to increase proportionately to immersion time with 74% mortality for 2 minutes, 84% for 5 minutes, and 87% for 10 minutes (Denny 2008). In

contrast, the mortality of mussels (*P. canaliculus*) was low, i.e., averaging from 0.9 to 1.6%. Freshwater immersion for 1–2 days achieved 100% mortality of the kelp, *Undaria pinnatifida*, without adversely affecting mussel health (Forrest and Blakemore 2006). The ascidian *Didemnum moseleyi* immersed in freshwater survived for up to 2 hours in winter but were dead after as little as 15 minutes in summer while the associated cultured Pacific oysters (*Crassostrea gigas*) were found to be unaffected (Katayama and Ikeda 1987).

Compatibility with hull and encapsulation material.
Freshwater has high compatibility with vessel hull infrastructure.

Feasibility: Economic viability, commercial availability, and regulatory compliance.

The use of freshwater pumped into the encapsulation area would likely present few barriers to approval in an encapsulation protocol. Furthermore, as an abundant resource there would be low cost involved and only logistic issues of acquisition and transport of the freshwater into the enclosure would remain.

Disposability

Freshwater used for osmotic shock will be releasable into the surrounding environment with a minimal risk.

Summary:

While osmotic shock has been shown to be effective at achieving very high mortality rates in select marine species, an ability to resist salinity fluctuations and extremes is a common trait among members of the marine biofouling community. The effectiveness of the use of freshwater in enclosure treatments may depend on the specific target species, as some species such as Mediterranean fanworm appear highly vulnerable to exposure to freshwater (Jute and Dunphy 2016). For broader taxonomic impact, a multistep process of freshwater application followed by the addition of biocide could be utilised to shorten effective timeframes for decontamination. However, it would need to be determined whether this will be more effective than the simple addition of a biocide to salt water in the enclosure system.

### Hydrogen sulphide or Sodium sulphite

Hydrogen sulphide is a poisonous, corrosive and flammable gas with the smell of rotten eggs. It is soluble in water and in solution is acidic. Sodium metabisulphide is used as a disinfectant, antioxidant, and preservative agent and has been used for the deoxygenation of cooling water systems for the control of zebra mussels, *D. polymorpha* (Morrisey and Woods 2015). Sodium sulphite at 118 mg l$^{-1}$ has been recommended for controlling freshwater pest species in New Zealand where it is has been approved by New Zealand's Environmental Protection Authority (Clearwater et al. 2008). In addition to the direct action against fouling organisms, sodium sulphite combines with oxygen to produce sodium sulphate, with no by-products or change in pH, and may

accelerate killing through anoxia. Extended periods of anoxia (up 12–25 days at > 20 °C) may be required to kill molluscs such as the zebra mussel and Asian clam (*Corbicula fluminea*), which makes it impractical for application for encapsulation systems (Matthews and McMahon 1995).

It is possible that microbial degradation or other processes lead to the production of sulphide compounds with the potential to be corrosive to metals and possibly other hull materials and coatings. Testing would be required to confirm that this type of biocide is both active against the spectrum of biofouling species in seawater conditions, and safe to use on the various vessel materials and coatings.

### Hydrogen peroxide

Hydrogen peroxide is another strong oxidiser that is commonly used as a bleaching agent and disinfectant. Solutions of 10-25 % are used to purify drinking water (Chattopadhyay et al. 2004). However, there are concerns about maintaining an effective dose due to rapid degradation to oxygen and water in seawater (Grandison et al. 2011). A 90 % mortality rate of adult zebra mussels (*D. polymorpha*) has been reported after exposure to 5.4 mg $l^{-1}$ for 21 days (Petrille and Miller 2000). The duration of treatment decreased with corresponding increases in hydrogen peroxide concentration, with 100% mortality of the zebra mussels also achieved at 7.8, 8.8 and 3.0 days for concentrations of 10, 20, and 40 mg $l^{-1}$ respectively. Asian clams (*Corbicula fluminea*) were observed to be even less sensitive to this biocide with 100% mortality observed after 13.5, 9.5 and 9 days at concentrations of 10, 20 and 40 mg $l^{-1}$ of hydrogen peroxide respectively.

No mortality of zebra mussels was observed after a 30 minute exposure to a concentration of 29.6 µg $l^{-1}$ (Matisoff et al. 1996). Compared to chlorine as a biocide, hydrogen peroxide is less effective, which would increase the application costs if higher concentrations were to be used to match effectiveness. However, hydrogen peroxide does not produce organo-halogenated compounds or other harmful decomposition products (Jenner et al. 1998). The timeframe needed to effect useful mortalities of target organisms in the studies done to date would indicate high concentrations of hydrogen peroxide would be needed, and the effect of these concentrations on vessel materials and coatings would need to be determined. Treatments may also require monitoring of the active concentration as hydrogen peroxide is known to degrade quickly in seawater. The probable impracticality of maintaining an effective concentration over a necessary period of time makes hydrogen peroxide an unlikely preference for encapsulation applications.

### Sodium metasilicate (Silicic acid)

Sodium silicate is used as an alum coagulant and an iron deflocculant in wastewater treatment, as well as insecticides, fungicides, and antimicrobial compounds. Denny (2008) tested a 6 % solution of sodium metasilicate with a mortality rate of about 70% after 2 minutes for *Didemnum vexillum*. It is strongly alkaline in solution and is noted in numerous chemical databases as corrosive to aluminium in its solid form. The lack of data against a full spectrum of biofouling species and the probable corrosive activity against hull materials make this an unlikely candidate for encapsulation applications.

### Iodine

Iodine is a halogen like chlorine and bromine which undergoes similar reactions in seawater. Chemically active paint systems for biofouling prevention consist often of a certain amount of cuprous iodine (Brooks and Waldock 2009). However, iodine has not been used much for water treatment technologies but is mentioned as a potential oxidant for biofouling control (Grandison et al. 2011). A commercial iodine injection system was used within cooling water systems of ships but was found to be less efficient than hypochlorite as a biocide acting against mussel fouling (Klassen et al. 2001).

### Ferrate

Potassium ferrate is a powerful oxidant. The reaction of ferrates with pollutants are typically fast, with the formation of non-toxic by-products (Sharma 2002). Ferrate is shown as environmentally safe and effective in controlling bacterial biofilm growth so that in model condenser systems, biofilm growth is substantially retarded (Fagan and Waite 1983). However, ferrate is relatively expensive with ferrate granules costing around NZ$12 per kilogram (Yates et al. 2014). A patented system for the onsite production of liquid ferrate is available but it requires precursor chemicals (sodium hydroxide, sodium hypochlorite, ferric chloride) that will require appropriate handling protocols (Grandison et al. 2011). Ferrate has not been tested yet to control marine biofouling (Growcott et al. 2016), and is an unlikely immediately useful candidate for encapsulation applications given the lack of knowledge of its use in this manner.

## Neutral particles

Neutral particles that encapsulate the biocides with nutrients may be a solution when ingested by filter feeders which is a predominant form of feeding among biofouling organisms. The principle is that the particles, in the size range of 40 to 250 µm, are identified as food by the filter feeder and are taken up and concentrated in the organism, whereupon the biocide is released and kills the organism. Aldridge et al. (2006) used a formulation containing potassium chloride, while Costa et al. (2011) used a formulation with unnamed proprietry biocides to kill freshwater the zebra mussel, *Dreissena polymorpha*, over 12 to 24 hour time periods. In the study of Aldridge et al. (2006) the mussels were continually treated with particles at 1 g $l^{-1}$ containing 300 mg $l^{-1}$ KCl, in a flume apparatus (water dimensions 4 m long x 5 cm wide x 5 cm deep) with a flow rate of 100 ml second$^{-1}$. A 12 hour treatment gave a kill rate of 60%, which was explained as being in line with the proportion of mussels that actively fed during the treatment period. It was suggested that repeat dosing would improve the overall kill rate of the mussels. Costa et al. (2012) published data showing the effectiveness of particles which incorporated polyquat biocide on zebra mussels.

The work published by Aldridge et al. (2006), Costa et al. (2011) and Costa et al. (2012) is all focussed on targeting the elimination of the unwanted freshwater zebra mussel. Aldridge et al. (2006) demonstrated that particles incorporating KCl were not harmful to a native unionid mussel

(*Anodonta anatina*) and discussed the likelihood that other non-target biota would remain unaffected when using KCl as a biocide. Costa et al. (2012) discussed this further and indicated that freshwater and saltwater mussel species (e.g., the blue mussel, *Mytilus edulis*), and other species of suspension feeders (e.g., sea squirts, sponges and bryozoans) would be susceptible to a neutral particle strategy, but research would be needed to define the best biocide cores and nutrient shells to target the species of interest.

In summary, neutral particles are commercially available and may offer a low toxicity, non-corrosive option for resilient filter feeders. The application of these particles to the encapsulation system would need to be tested, possibly optimised for the range of unwanted target species, and the cost and supply of application doses would also need to bear careful consideration.

**Chemical Relaxants and Anaesthetics**

A wide range of chemical relaxants and anaesthetics are routinely used for the handling of marine invertebrates prior to preservation and during aquaculture operations (Norton et al. 1996, Biological Bulletin Compendia 2018). These chemicals include propylene phenoxetol, 2-phenoxyethanol, menthol crystals, menthol liquid, clove oil, benzocaine, MS222, chloral hydrate, sodium pentobarbitone, magnesium chloride, sodium bicarbonate, carbon dioxide gas, ketamine-HCl, isobutyl alcohol, chloroform and many more. The effectiveness of individual chemicals varies enormously among taxa, presumably in response to the presence of different metabolic pathways. Likewise, the dose of individual chemicals can produce varying levels of anaesthesia from muscle relaxation through to extensive metabolic depression and arrest. While it may be challenging to use such chemicals to act directly as biocides, the responses they elicit may be particularly helpful in improving the effectiveness of other biocides by improving access of the biocide to tissues. For example, resilient biofouling organisms that use closure mechanisms to avoid contact with seawater treated with biocides (e.g., barnacles, tubeworms, gastropods and bivalves) could potentially be chemically relaxed prior to the introduction of the biocide to prevent their closure via inactivating their closure muscles or blocking their sensory warning systems used in early detection of biocides. The more chemically complex agents, such as benzocaine, MS222, ketamine-HCL, sodium pentobarbitone tend to be more expensive, have issues with safe handling and disposal. However, a number of these chemical agents are low cost, readily available, safe to handle and to dispose of into the environment, including menthol (i.e., the primary constituent of peppermint oil), clove oil and magnesium chloride. The use of these chemical agents in conjunction with biocides has not been reported in any detail, but warrants further investigation as their use has the potential to overcome resistance of some hull biofouling species to otherwise effective biocides. The possible use of step-wise treatments in enclosure systems may require more detailed monitoring of concentrations of active chemicals and modifications to the systems used for the delivery of the chemicals to the enclosures.

**1.3 Non-chemical treatment methods**

Non-chemical treatments aim to exceed the tolerance of biofouling organisms to a given environmental parameter. Compared to chemical treatment agents, fewer toxicological and environmental risks exist for non-chemical treatment agents. Growcott et al. (2016) identified four non-chemical treatments that could be applied to vessel pipework: physical removal, thermal stress, deoxygenation, and osmotic shock. Non-chemical treatments are separated into physical or mechanical treatment methods and have been mainly developed for the treatment of ballast water, and wastewater and internal vessel pipework (Chattopadhyay et al. 2004, De Lafontaine et al. 2009, Gregg et al. 2009, Tsolaki and Diamadopoulos 2010, Werschkun et al. 2014, Lloyd's Register Marine "Understanding ballast water management - Guidance for shipowners and operators" 2015, Nosrati-Ghods et al. 2017).

Ballast water is not only treated against potential invasive fouling species but also in general for many microorganisms, comprising phytoplankton, zooplankton and microbial (Tsolaki and Diamadopoulos 2010, Werschkun et al. 2014). Some of the ballast water treatment methods might also be considered for use in encapsulation treatment.

No single in-water technique has been shown to effectively remove all potentially unwanted or invasive species, therefore flexible and multi-technique approaches are typically taken (Gregg et al. 2009). Physical methods in ballast water treatment mainly include separation of suspended living particles through filtration and hydro cyclones, and are likely to be of little use for treatment of biofouling in encapsulation systems. Mechanical and chemical treatment methods involve de-oxygenation, UV radiation, thermal treatment, and pH adjustment, combined with biocidal chemicals such as chlorine, ozone, hydrogen peroxide and chlorine dioxide (Gregg et al. 2009, Tsolaki and Diamadopoulos 2010, Werschkun et al. 2014, Nosrati-Ghods et al. 2017).

The mechanical methods, UV radiation and heat treatment, might be used in combination with biocides for the encapsulation treatment systems, serving to potentially increase effectiveness and shortening treatment time (Gregg et al. 2009, Bracken et al. 2016). However, this would need a more detailed cost and logistical analysis for feasibility depending on the hull structure requiring treatment, and if it is practical to use these alternative methods in lieu of biocides.

In wider terms, many of the above-mentioned techniques are yet to be determined as applicable to an encapsulation scenario. Specific research will be required to determine effectively whether they provide a feasible and adequate alternative to the addition of biocides within an encapsulation treatment system.

### De-oxygenation

Numerous studies report the encapsulation process on various structures, including marine vessels, as successful against biofouling organisms without the addition of a specific biocide, albeit with longer treatment durations (Coutts and Forrest 2005, Coutts and Forrest 2007, Inglis et al. 2012,

Atalah et al. 2016). In these instances it is reported as essential to ensure there are no failures in the enclosure system if the intended outcome is 100% mortality of a target species.

De-oxygenation in ballast water is artificially created with varying success by the addition of nutrients, such as glucose, or a reducing agent such as a sulphide, or the injection of inert gases, or by the use of a vacuum chamber (Gregg et al. 2009). However, these approaches are designed only to accelerate the development of hypoxia and have not been tested in terms of encapsulation systems. A review of ballast water treatments infers that regardless of the effectiveness of these various ballast water control methods they all could be enhanced to near 100% kill rates with the addition of a biocide (Gregg et al. 2009).

Effectiveness: Biocidal spectra and time frames:

The combination of low oxygen (2%) and high carbon dioxide (12%) is capable of eliminating >95% of zooplankton and invertebrates within several hours, and can kill >99% of *Vibrio cholerae* in 24 hours (Tsolaki and Diamadopoulos 2010). Wrapped wharf piles needed to be left for up to 7 days to completely kill all polyps of the invasive corals *Tubastraea tagusensis* and *T. coccinea* (Mantelatto et al. 2015)*.* The effect on encapsulation with and without a biocide was tested on two model organisms, ascidians *Ciona* spp. and mussels *Mytilus galloprovincialis* (Atalah et al. 2016). It was found that *Ciona* was far less resilient to encapsulation than *Mytilus galloprovincialis*, and total mortality was achieved within 24 hours, whereas for *M. galloprovincialis* it took up to 18 days. It was also shown that at a higher temperatures and with the presence of larger amounts of biofouling biomass, oxygen depletion, sulphide accumulation and the mortality of biofouling occurred more quickly.

Encapsulation studies have revealed that some biofouling species, especially bivalves and bryozoans, are particularly resistant to hypoxia for 10 days or longer (Pool et al. 2013, Atalah et al. 2016). Additionally, black encapsulation sheets should be used to prevent any photosynthesis, which would otherwise support photosynthesising biofoulers and serve to counteract the hypoxic conditions. In various cases, longer treatment periods have increased the risk of enclosure materials being torn or breaking loose so that the hypoxic effect of the enclosure is lost. Thus, careful installation of enclosures, selection of suitable sites to conduct the enclosure (i.e., low water movement) and careful monitoring for continued integrity of the enclosure are essential.

Compatibility with hull and encapsulation material

No chemical addition is an ideal solution to management of biofouling on vessel hulls or other such structures that may be incompatible with biocides. As de-oxygenation methods require complete quarantine conditions, sharp edged shells of various biofouling species and contact against docks or other such structure may interfere with the integrity of the wrap over the extended time periods required for this technique.

Feasibility: Economic viability, commercial availability, and regulatory compliance

Using enclosure with hypoxia treatment of biofouling has been described as equivalent in cost to hauling out and water blasting the hulls of vessels of <20 m length, which can be achieved much faster than hypoxic mortality (Inglis et al. 2012). The availability of suitable wrapping materials and trained divers for installation, as well as the increased timeframe for 100% mortality appear to be the main factors limiting viability of this enclosure method using hypoxia. However, the absence of a haul out facility, or where the use of biocides has not been approved, enclosure using hypoxia may provide a viable alternative treatment option.

Disposability

While no chemical disposal would be necessary in a hypoxic treatment, disposal of organic material and subsequent compounds from breakdown of organic material may still require some consideration, especially where it is possible that live biofouling organisms may remain among the material. This issue may also apply where chemical biocides are used.

Summary:

While hypoxia remains a proven method at reducing or eliminating biofouling taxa, it comes at the cost of much extended timeframes. Obvious ramifications of this extended timeframe include the susceptibility of the enclosure material to damage which may compromise the treatment. While no chemical additions are required, it may still be necessary to remove dead and sheared organic material from the treatment site, or neutralise any further risk of contaminant spread to the treatment area. Whether or not the addition of hypoxia inducing chemicals is additive to the effectiveness of de-oxygenation methods, or helps to decrease the timeframes for these treatments is yet to be understood, as it has not yet been tested in an encapsulation scenario.

## **Ultraviolet (UV) Radiation**

UV radiation is mainly used for disinfection of waste, surface and ballast water by destroying bacteria and viruses (Gregg et al. 2009, Tsolaki and Diamadopoulos 2010, Bracken et al. 2016). UV radiation operates by causing photochemical reactions with biological components such as nucleic acids (DNA and RNA) and proteins. However, the effectiveness of UV treatment depends on the size and type of organisms and sufficient exposure to the radiation. An ultraviolet dose of 563 mJ cm$^{-2}$ was used to target zooplankton species, *Nereis virens, Acartia tonsa, Tisbe battagliai, Alexandrium tamarense* and *Thalassiosira pseudonan,* however, only 56% of the zooplankton could be eradicated (Tsolaki and Diamadopoulos 2010). A UV dosage of 200 mWs cm$^{-2}$ gave a 95% kill of zooplankton, while phytoplankton levels were reduced, but bacterial levels increased, probably in response to the levels of decomposing organic matter (Wright et al. 2004).

Therefore, different UV dosages are required to kill a range of different organisms, such as bacteria, protozoa, microalgae and zooplankton (Gregg et al. 2009). Hence, UV radiation is best

used in combination with other treatments such as the addition of chemicals to reach a more complete eradication of micro- and macro-organisms.

The complete irradiation of any significantly sized hull structure with UV is problematic. While UV sterilisation of moving water, such as in a ballast water or pipe system, is attainable, any treatment to extensive surfaces across a stationary vessel involves obvious practical difficulties.

## Heat treatment (thermal)

For ballast water, excess heat produced by the vessel's engines or from the boiler system can be used to treat the water, providing an energy efficient source rather than having to manually input heat to the system, as would be the case for encapsulation (Gregg et al. 2009). The minimum temperature needed to deactivate unwanted fouling species is over 40 °C. However, for vegetative bacteria, fungi and viruses much higher temperatures up to 55 °C are needed to kill 95% of the bacteria but not their spores (Gregg et al. 2009).

Heat treatments have been used previously to treat fouling on vessel hulls (Wotton et al. 2004) by using underwater flame torches that heated small segments of the water around the vessel to 70 °C to remove *Undaria pinnatifida* sporophytes present on the hull. The treatment was reportedly highly expensive and time consuming. Deployable and variable output heating elements that provide in water heat treatment options have been patented, however, they do not provide for complete hull coverage and may require lengthy treatment periods to ensure 100% elimination of biofouling.

Similarly to UV radiation, a heat treatment protocol in situ for even a small vessel would be difficult to implement while maintaining adequate temperatures to attain 100% kill. Insulation of the wrap material might be required in testing of this technique to prevent substantial heat loss to the colder ambient temperature outside the encapsulation. In combination with a biocide, this method may prove effective as a direct application against biocide resistant taxa.

## pH adjustment

Increasing or decreasing pH can be achieved by the addition of alkali or basic chemicals and may effectively kill many organisms (Gregg et al. 2009, Tsolaki and Diamadopoulos 2010). However, changing the pH may increase corrosion and create toxic by-products or radical molecules depending on the chemical source. This method is likely to be rather costly and might result in the production of harmful by-products. Obtaining sufficient shifts in pH to provide efficacy against biofouling organisms would potentially involve substantial quantities of acid or base which are likely to raise transport and handling concerns.

**KNOWLEDGE GAPS**

It is apparent from reviewing the available information on the use of biocides for the treatment of biofouling that much of our current knowledge comes from reported results from numerous piecemeal studies, which are limited in their scope by the specific situational circumstances, breadth of taxa, extent of the biofouling being treated, or the specifics of biocidal application (i.e., biocide, and strength and duration of dose). This makes it more difficult to assess the efficacy of any particular biocide, especially when used in a situation, like in-water enclosure treatment, for which there has been relatively little prior research. For example, the effectiveness of biocides are frequently reported from tank studies using individuals of a single species that have been relocated from the wild and applied in filtered seawater, a situation likely to produce results that are different to in situ application of the same biocide.

The haul out of a vessel followed by hull scraping and/or pressure washing or blasting, can be expected to provide the most reliable elimination of all hull biofouling, especially if pipework is also drained and dried. It is clear from reviewing the available information that evidence is lacking that this level of effectiveness is matched by in-water enclosure and encapsulation methods for the treatment of marine biofouling on vessel hulls. Results of previous studies and practical observations suggest that some hard-bodied taxa are resistant to, and can survive, current biocidal treatments most commonly used in-water enclosure and encapsulation methods, such as chlorine.

Given the wide acceptance and current use of chlorine as a biocide for in-water enclosure treatment, the ability of resilient hull biofouling taxa to survive exposure to this biocide, especially those of specific concern, should be further determined to provide greater confidence in the breadth of biosecurity benefits this intervention can provide.

Perhaps more importantly, the potential for improving the effectiveness of chlorine for this application, especially against resilient taxa, through the use of combinatorial treatments warrants further investigation. For example, the application of muscle relaxants or low toxicity aquatic anaesthetics (e.g., magnesium chloride or clove oil) could be explored as a means of overcoming the resistance of biofouling animals capable of closure to avoid the toxic effects of biocides.

Ideally these studies would be completed in situ, using practical application of in-water enclosure systems. Likewise, the circumstances in which biocides are applied in enclosure systems and the corresponding results need to be documented, as it is highly likely that many variables may also impact on the effectiveness of biocides. These variables include, the biofouling taxa, their origins (e.g., tropical waters), the extent of fouling, features of the hull (e.g., presence of seachests), water temperature at time of treatment, and quality of the seawater in the enclosure. A better understanding of the importance of these variables would ultimately lead to improved treatment protocols, or customised protocols to meet specific treatment situations or to deal with more resilient taxa when identified as being present.

The interaction of biocides with vessels is poorly documented, and relies heavily on casual observations of those involved in prior application of in-water enclosure treatments. Chlorine is chemically active in seawater and may interact with some biofouling coatings and will interact with other infrastructure, such as protective zinc electrodes. Such interactions may have the potential to exacerbate biofouling concerns, such as through the inactivation of remaining

antifouling coatings on hulls. Further investigation of the effect of chlorine biocide on vessel infrastructure is warranted if this method of vessel treatment is to be used more extensively.


## CONCLUSIONS AND RECOMMENDATIONS

In-water enclosure and encapsulation methods for the treatment of marine biofouling on vessel hulls has recently become an important additional tool for improving marine biosecurity in New Zealand. Although not extensively used, these treatment methods have significant value for intervention in remote locations, emergency situations, and for vessels that are difficult to haul out.

Given the recent and emerging nature of in-water enclosure and encapsulation methods for the treatment of marine biofouling on vessel hulls there are a limited number of comprehensive studies on their effectiveness. Early adopters of the technology have self-selected granulated chlorine as the biocide of choice, largely for practical reasons rather than any comprehensive assessment of biocidal efficacy. This selection of chlorine as a preferred biocide has been reinforced by regulatory acceptance of the use of granulated chlorine for this purpose, and the discharge of the treatment products to the marine environment.

Observations from practical application and the results from studies indicate that whilst chlorine is highly effective against soft-bodied biofouling organisms, it is less so for those biofoulers capable of self-enclosure, such as bivalves and slipper limpets. Such organisms are among our existing marine NIS, and others with the potential to establish (e.g., green mussel, *Perna viridis*) pose an ongoing risk of establishing in New Zealand waters as a result of introduction via vessel hulls.

Acetic acid has shown a degree of effectiveness against some biofoulers capable of self-enclosure, with the acid capable of eroding the calcareous shells to exposure the living tissues to the biocide. Studies show that acetic acid is also a consistently effective biocide for many soft-bodied biofouling species. While acetic acid is readily commercially available and relatively inexpensive, the storage and handling of this acid poses significant risks, especially for larger quantities which would be required for use in vessel enclosure treatments. In contrast, chlorine products are relatively safer to store and handle, while also being readily available and relatively inexpensive.

Further research is recommended to quantify and improve the effectiveness of the use of chlorine as a biocide when used for in-water enclosure and encapsulation methods for the treatment of marine biofouling on vessel hulls. In particular, examination of combinatorial chemical treatments is warranted, particularly for those biofouling taxa that are resistant to the conventional chlorine treatment.

Further research to determine how the effectiveness of in-water enclosure and encapsulation methods is influenced by other variables, such as the biofouling taxa, the overall extent of biofouling, and features of the hull. More comprehensive monitoring of the effectiveness of existing enclosure treatment operations, would also assist in this regard and contribute to incremental improvements of the operation of these systems.

Table 1: Potential biocides which are already commercially used, have been experimentally tested in the laboratory or field, or a theoretically mentioned in the literature.

| Biocide | Study | Concentration used | Exposure time | Species | Mortality | Study | Compatibility | Feasibility | Disposal |
|---|---|---|---|---|---|---|---|---|---|
| Acetic Acid | Forrest et al 2007 | 4 % | 1 to 4 minutes | Soft bodied organisms | 100 % | Field trial on fouled ropes | No problems noted | 5 % acetic acid is vinegar, which has no regulatory barriers to use.<br><br>Problems are apparent with the use of concentrated acetic acid. Reasonable cost and ease of supply. | Direct release or release after neutralisation with sodium hydroxide. |
| | | | 1 to 4 minutes | Calciferous species | <10 % | | | | |
| | | | 4 minutes | *Ciona intestinalis* | 100 % | | | | |
| | | | 4 minutes | *Hydroides elegans* | survived | | | | |
| | Lewis & Dimas 2007 | 0.2, 0.4 % and 2 % | 6, 14 and 48 h | *Mytilus galloprovincialis planulatus* | 100 % kill required 14 h and 0.4 %. | Laboratory study | | | |
| | Carver et al 2003 | 5 % | 30 seconds | *Ciona intestinalis* | 100 % | Laboratory study | | | |
| | Rolheiser et al. 2012 | 0.25, 1.25 and 5 % | 1, 5 and 10 minutes | *Crassostrea gigas* | 100 % kill required 5 % for at least 5 minutes | Field trial | | | |
| | | | | *Didemnum vexillum* | 100 % kill required 5 % for at least 5 minutes | | | | |
| | McCann et al. 2013 | 10 % | 10 minutes | *Didemnum vexillum* | 100 % | Field trial | | | |
| | Piola et al. 2009a | 5 % | 1 minute exposure | *Didemnum vexillum* | 100 % | Spray application trial. Sprayed and left unrinsed. | | | |
| | | 5 % | 30 minutes | *Botrylloides leachi* | 75-100 % | | | | |
| | | 5 % | 30 minutes | *Botrylloides schlosseri* | 75-100 % | | | | |
| | | 20 % | 12 h | *Bugula neritina* | survived | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5 % | 30 minutes | *Ciona intestinalis* | 75-100 % | | | | |
| | Piola & Conwell 2010 | 5% | < 24 h | *Didemnum vexillum* | 100 % | | | | |
| | Roche et al. 2015 | 5 % | 30 minutes | *Didemnum vexillum* | 81% (reduction in biomass after 2 weeks) | Laboratory study | | | |
| | | | 15 minutes | | 60 % (surface area of colony removed) | Encapsulation study | | | |
| | Coutts & Forrest 2005 | 1 % | 10 minutes | *Styela clava* | 100 % | Field trial | | | |
| | | 1% | 12 h | *Crassostrea gigas* | survived | | | | |
| | | 1 % | 12 h | *Pomatoceros terraenovae* | survived | | | | |
| | Denny 2008 | 4 % | 10 minutes | *Didemnum vexillum* | 87 % | Laboratory study | | | |
| | | 4% | 10 minutes | *Didemnum vexillum* | >90 % | Field trial | | | |
| | Locke et al. 2009 | 0.032 % | 4 days | Three spine stickleback | 100 % | Immersion studies | | | |
| | | 0.05 % | 4 days | Sand shrimp | | | | | |
| | Pannell & Coutts 2007 | 5 % | 7 days | *Didemnum vexillum* | 100 % | Encapsulation | | | |
| | Atalah et al 2016 | 5 % | 48 h | Established field communities | 100 % | Encapsulation study | | | |
| Chlorine | Piola et al. 2009a | 20,000 mg/L | 6 h | *Botrylloides leachi* | 75-100 % | Spray application trial. Sodium Hypochlorite. | No problems noted in encapsulation and other studies. | No regulatory barriers to use of hypo- | Neutralised with sodium thiosulphate before disposal. |
| | | 10,000 mg/L | 6 h | *Botrylloides schlosseri* | 75-100 % | | | | |
| | | 20,000 mg/L | 12 h | *Bugula neritina* | Survival | | | | |

| Reference | Concentration | Time | Species | Efficacy | Method/Notes | | Comments | |
|---|---|---|---|---|---|---|---|---|
| | 20,000 mg/L | 30 minutes | *Ciona intestinalis* | 75-100 % | | | chlorites or dichlor. | |
| Williams & Schroeder 2004 | 125 mg/L | 30 minutes | *Caulerpa taxifolia* | 100 % | Laboratory study. Sodium hypochlorite | | Solid nature of Calcium hypochlorite and dichlor makes storage and application easier; both also have the best FAC generation. | |
| Thompson & Richardson 2010 | 100 mg/L | 48 h | *Cerastoderma edule* | 100 % | Laboratory study. Sodium hypochlorite | | | |
| | 1000 mg/L | 5 minutes | | | | | | |
| Denny 2008 | 0.25 % | 2 minutes | *Didemnum vexillum* | 100 % | Laboratory study. Dipping in diluted bleach. | | | |
| Pannell & Coutts 2007 | 200 mg/L | 12 h | *Didemnum vexillum* | 100 % | Encapsulation. Sodium hypochlorite. | | Reasonable cost and availability. | |
| Roche et al. 2015 | 1 % | 30 minutes | *Didemnum vexillum* | 80 % reduction in colony size | Laboratory. Sodium hypochlorite. | | | |
| | 500 mg/L | 4 x 15 minutes | | 100 % | Encapsulation. Sodium hypochlorite. | | Chlorine dioxide has higher cost with on-site generation and safety issues. | |
| Holt and Ryan 1997 | 5 mg/L | 70 minutes | *Dreissena polymorpha* | 100 % | Laboratory. Chlorine dioxide. | | | |
| Matisoff et al. 1996 | 40 mg/L | 6 minutes | *Dreissena polymorpha* | 100 % | Laboratory. Chlorine dioxide. | | | |
| Rajagopal et al. 2002 | 0.25 mg/L | 89 to 109 days | *Mytilopsis leucophaeata* | 100 % | Field trial. Time to kill was dependent on size. | | | |
| Bax et al. 2002 | 12 mg/L | 111 h | *Mytilopsis sallei* | 100 % | Laboratory test, Calcium hypochlorite. | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10 mg/L | After 6 days | | Substantial reduction, but eradication failed. | Field use to eradicate invasive species in marinas. | | | |
| Masilamoni et al. 2002 | 9.1 mg/L | 114 h | *Perna viridis* | 100 % | Field use in cooling tower water. Sodium hypochlorite. | | | |
| Rajagopal et al. 1995 | 10 mg/L | 48 h | *Perna viridis* | 100 % | Laboratory study. Sodium hypochlorite. | | | |
| Rajagopal et al. 2003 | 10 mg/L | 48 h | *Perna viridis* | 100 % | Laboratory study. Sodium hypochlorite. | | | |
| Morrisey et al 2016 | 200 mg/L FAC | 16 h | *Sabella spallanzanii* | 100 % | Encapsulation. Dichlor. | | | |
| Coutts & Forrest 2007 | 50 mg/L FAC | 6 h | *Styela clava* | 100 % | Laboratory study. | | | |
| Coutts & Forrest 2007 | 200 mg/L starting FAC | 12 h | *Asterocarpa cerea* | survival | Encapsulation field trial on pontoons. | | | |
| | 200 mg/L starting FAC | 12 h | *Codium fragile* | survival | Encapsulation field trial on pontoons. | | | |
| | | | *Crassostrea gigas* | survival | | | | |
| | | | *Crepidula costata* | survival | | | | |
| | | | *Ecklonia radiata* | survival | | | | |
| | | | *Pomatoceros terraenovae* | survival | | | | |
| | | | *Styela clava* | 100 % where final FAC was >4 mg/L | | | | |
| | | | *Styela picata* | survival | | | | |

| Quaternary Ammonium Compounds | Garaventa et al. 2012 | 1000 mg/L | 72 h | *Brachidontes pharaonis* | 100 % | Laboratory test. Only the effective QAC biocide is shown. Others were tested without eradication of the target species. | No problems noted for internal pipework and sea chests. Some QAC are labelled as corrosive. | Commonly used biocides with well-established protocols. Mid-range costs, commercially available. | Some uncertainty as effective treatments have exceeded European discharge limits. |
|---|---|---|---|---|---|---|---|---|---|
| | Costa et al. 2012 | 800 mg/L | 12 h | *Dreissena polymorpha* | 100 % | Laboratory. | | | |
| | Britton & Dingman 2011 | 3 % | 60 minutes | *Dreissena rostiformis begnsis* | 100 % | Laboratory. | | | |
| | Lewis & Dimas 2007 | 1 % | 6 h | *Mytilus galloprovincialis planulatus* | 100 %. But not as effective as acetic acid in parallel trials. | Laboratory. | | | |
| | Neil & Stafford 2005 | 10 % | 12 h | *Saccostrea glomerata* | 10-20%. Not as effective as acetic acid in parallel trials. | Laboratory. | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Piola & Grandison 2013 | 5 % | 24 h | *Mytilus galloprovincialis planulatus* | 100 % | Internal pipework field trial. | | | |
| Bromine | Chattopadhyay et al. 2004 | 0.1 to 10 mg/L | Hours (low concentrations). Minutes (high concentrations). | Bacteria, Zooplankton, fish, water fleas | 100% | Cooling waters field trial. | Reported as corrosive. | Mid-range cost. Concerns over toxicity. | Environmental toxicity may limit disposal. |
| | WHO 2018 | 0.52 mg/L | 24 h | *Lepomis macrochirus* | 100% | Laboratory. | | | |
| | | 0.31 mg/L | 24 h | *Oncorhynchus mykiss* | 100% | | | | |
| | | 1.5 mg/L | 24 h | *Daphnia magnamagna* | 100% | | | | |
| | | 1 mg/L | 48 h | *Daphnia magnamagna* | 100% | | | | |
| Calcium (hydr)oxide (lime) | Carver et al 2010 | 4 % | 10 minutes | *Ciona celata* | 32 % | Laboratory. | Reported as corrosive. | Reasonably low cost and commercially available. | No problems noted, may have positive effects. |
| | | | | *Crassostrea virginica* | 0 % | | | | |
| | Rolheiser et al 2012 | 4 % | 30 minutes | *Crassostrea gigas* | 60 % | Laboratory. | | | |
| | | 4 % | 10 minutes | *Didemnum vexillum* | 100 % (as coverage) | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 4 % | 5 minutes | *Evasterias troschellii* | 100 % | | | | |
| | Piola et al. 2009a | 20 % | 6 h | *Botrylloides leachi* | 100 % | Spray application trial. | | | |
| | | | 12 h | *Botrylloides schlosseri* | 100 % | | | | |
| | | | 12 h | *Bugula neritina* | survival | | | | |
| | | | 12 h | *Ciona intestinalis* | 100 % | | | | |
| | | | 12 h | *Watersipora subtorquata* | 100 % | | | | |
| | Denny 2008 | 10 % | 2 minutes | *Didemnum vexillum* | 100 % | Laboratory study. | | | |
| Copper Sulphate | Bax et al. 2002 | 4 mg/L in combination with sodium hypochlorite | | *Mytilopsis sallei* | 100 % | Field use to eradicate invasive species in marinas. | No problems reported. | Reasonable cost and commercially available. May be regulatory issues because of environmental toxicity and bioaccumulation. | Environmental toxicity may limit disposal. |
| | Ferguson 1999 | 4 mg/L in combination with sodium hypochlorite | | *Mytilopsis sallei* | 100 % | Field use to eradicate invasive species in marinas. | | | |
| Descalers, HCl-based | Bracken et al. 2016 | 1 in 4 dilution | 8 h | *Mytilopsis sallei* | 70 % (as biomass) | Laboratory study. | Likely to be corrosive at the | May be costly at the concentrati | Long term environmental effects are not known. |
| | Lewis & Dimas 2007 | 1 in 4 dilution | 24 h | *Mytilopsis sallei* | 100 % | Laboratory. | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Neil & Stafford 2005 | 1 in 4 dilution | 12 h | *Saccostrea glomerata* | 100 % | Laboratory. | concentrations required. | ons required. | |
| Ferrate | Fagan & Waite 1983 | 10 µM | Dosing 2 × a day for 5 minutes. | Bacterial biofilms. | 100 % retardation of growth. | Laboratory. | Unknown. | A more costly option, but commercial preparations are available. | Reported as being environmentally safe. |
| Hydrogen peroxide | Petrille & Miller 2000 | 40 mg/L | 9 days | *Corbicula fluminea* | 100 % | Laboratory. | May have an effect. | May be difficult to maintain an effective dose. | Will breakdown quickly to water and oxygen. |
| | | | 3 days | *Dreissena polymorpha* | 100 % | Laboratory. | | | |
| | Matisoff et al. 1996 | 29.6 µg/L | 30 minutes | *Dreissena polymorpha* | 0 % | Laboratory. | | | |
| Iodine | Klassen et al. 2001 | Commercial Iodine injection system. Concentration not stated. | | mussels | 80 % reduction in colonisation of pipework, compared to 100 % inhibition of colonisation for sodium hypochlorite. | Model pipework. | Powerful oxidant, may cause damage. | Less effective and more expensive than other systems. | No specific information. |
| Ozone | Jones et al 2006 | 0.28 mg/L/minute | 90 minutes | Mysid shrimp | 100 % after 48 h | Ballast water trials | Likely to be corrosive at | Unlikely. | Breaks down rapidly, but |

| Treatment | Reference | Concentration | Time | Organism | Effect | Setting | Corrosiveness | Marine use | Environmental |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Sheepshead minnow | Lethal toxicity of 50 % of organisms occurs in half the time noted for mysid shrimp. | Ballast water trials | the concentrations required. | | may form toxic by-products in sea water. |
| | Viera et al 1999 | 0.3 mg/L | 30 minutes | Bacteria | 100 %, $10^8$ CFU/mL. | Laboratory. | | | |
| Peracetic acid | De Lafontaine et al. 2009 | Commercial preparation . 100 mg/L. | 48 h | Bacteria | 100 % | Laboratory. | Likely to be corrosive at concentrations required. | Unlikely. | Breaks down to non-toxic by-products, but long term environmental effects not known. |
| | | | | *Dreissena polymorpha* | 0 % | | | | |
| | Verween et al. 2009 | Commercial preparation.150. 5 mg/L. | 15 minutes | *Dreissena polymorpha* | 100 % | Laboratory. | | | |
| | | | | *Mytilopsis leucophaeata* | 100 % | | | | |
| Sodium hydroxide (caustic soda) | Kerrison et al. 2016 | pH 12.5 | 10 minutes | Crustaceans, Rotifers, Green algae | 100 % | Laboratory. | Likely to be corrosive at concentrations required. | Concentr-ations required for marine NIS may limit use. | Not known for the concentrations required. Can be neutralised before disposal. |
| | Denny 2008 | 6 % | 2 minutes | *Didemnum vexillum* | 100 % | Laboratory. | | | |
| | TenEyck 2009 | pH 12 | On boat system | Crustaceans, Rotifers, Green algae | 100 % | Ballast waters | | | |

| | Montresor et al 2013 | 88.5 µg/L | 96 h | *Limnoperna fortunei* | LC$_{50}$ | Laboratory. | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sodium metasilicate (silicic acid) | Denny 2008 | 60 g/L | 2 minutes | *Didemnum vexillum* | 70% | Laboratory. | Likely to be corrosive at concentrations required. | Unknown. | Unknown. |
| Freshwater (osmotic shock) | Dunphy et al. 2005 | | 5 h | Oysters and mussels | Survival | Laboratory. | Unlikely to have a deleterious effect. | Will need to remove salt water during application. | Disposal will have minimal risk. |
| | | | | *Boccardia acus* | >98 % | | | | |
| | | | | *Sabella spallanzanii* | >98 % | | | | |
| | Jute & Dunphy 2016 | | 2 h | Oysters and mussels | Survival | Laboratory. | | | |
| | | | | *Sabella spallanzanii* | 100 % | | | | |
| | Kateyama & Ikeda 1987 | | 15 minutes in summer | Oysters and mussels | Survival | Laboratory. | | | |
| | | | | *Didemnum moseleyi* | 100 % | | | | |
| | Forrest & Blakemore 2006 | | 2 days | *Undaria pinnatifida* | 100 % | Laboratory. | | | |

| | Denny 2008 | | 10 minutes | *Didemnum vexillum* | 87 % | Laboratory. | | | |
|---|---|---|---|---|---|---|---|---|---|

## References

Aldridge, D. C., Elliott, P., Moggridge, G. D. (2006). Microencapsulated BioBullets for the control of biofouling zebra mussels. *Environmental Science and Technology* 40(3): 975-979.

Anderson, L. W. J. (2005). California's Reaction to *Caulerpa taxifolia*: A model for invasive species rapid response. *Biological Invasions* 7(6): 1003-1016.

Aquenal (2009). A yacht enclosure system to mitigate biosecurity risks associated with biofouling (Developed IP Report) MAF Biosecurity New Zealand Paper No: 2009/41 Prepared for MAF Biosecurity New Zealand.

Ashraf, M. A., Ullah, S., Ahmad, I., Qureshi, A. K., Balkhair, K. S., Abdur Rehman, M. (2014). Green biocides, a promising technology: current and future applications to industry and industrial processes. *Journal of the Science of Food and Agriculture* 94(3): 388-403.

Atalah, J., Brook, R., Cahill, P., M. Fletcher, L.M., Hopkins, G. A. (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. In: Veitch, C. R., Clout, M. N. *Turning the tide: the eradication of invasive species*, IUCN SSC Invasive Species Specialist Group, IUCN, Switzerland and Cambridge, UK. Page 26-39.

Biological Bulletin Compendia (2018) Invertebrate anesthetics and relaxants: Agents and organisms. Table 1. Accessed from: http://comm.archive.mbl.edu/BiologicalBulletin/ANESCOMP/AnesComp-Tab1.html

Borkow, G., Gabbay, J. (2005). Copper as a biocidal tool. *Current Medicinal Chemistry* 12(18), 2163-2175.

Bracken, J., Gust, N., Ross, J., Coutts, A. (2016). An assessment of the efficacy of chemical descalers for managing non-indigenous marine species within vessel internal seawater systems and niche areas. *Management of Biological Invasions* 7(3), 241-256.

Britton, D., Dingman, S. (2011). Use of quaternary ammonium to control the spread of aquatic invasive species by wildland fire equipment. *Aquatic Invasions* 6(2), 169-173.

Brooks, S., Waldock, M. (2009). The use of copper as a biocide in marine antifouling paints. Advances in Marine Antifouling Coatings and Technologies, Woodhead Publishing Limited, Cambridge, UK. pp. 492-521.

Cabrera, C., Gabaldón, C., Marzal, P. (2005). Sorption characteristics of heavy metal ions by a natural zeolite. *Journal of Chemical Technology and Biotechnology* 80(4): 477-481.

Cahill, P., Hickey, C., Lewis, P., Tait, L., Floerl, O. (2018). Treatment agents for biofouling in internal pipework of recreational vessels. MPI Technical Paper No: 2018/ In review.

Carlton, J. T., Geller, J. B. (1993). Ecological roulette: The global transport of nonindigenous marine organisms. *Science* 261: 78-82.

Carpenter, J. F., Nalepa, C. J. (2005). Bromine-based biocides for effective microbiological control in the oil field. In: SPE International Symposium on Oilfield Chemistry. Society of Petroleum Engineers. The Woodlands, Texas.

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

Carver, C. E., Thériault, I., Mallet, A. L. (2010). Infection of cultured Eastern oysters, *Crassostrea virginica*, by the boring sponge, *Cliona celata*, with emphasis on sponge life history and mitigation strategies. *Journal of Shellfish Research* 29(4): 905-915.

Chattopadhyay, S., Hunt, C. D., Rodgers, P. J., Swiecichowski, A. L., Wisneski, C. L. (2004). Evaluation of biocides for potential treatment of ballast water. Battelle Memorial Institute Report No. CG-D-01-05. 149 pp.

Clearwater S. J., Hickey C. W., Martin, M. L. (2008). Overview of potential piscicides and molluscicides for controlling aquatic pest species in New Zealand. Report #283. Science & Technical Publishing, Department of Conservation, Wellington. 74 pp.

Cortés, C., Marcos, R. (2018). Genotoxicity of disinfection byproducts and disinfected waters: A review of recent literature. *Mutation Research* 831:1-12.

Costa, R., Aldridge, D. C., Moggridge, G. D. (2011). Preparation and evaluation of biocide-loaded particles to control the biofouling zebra mussel, *Dreissena polymorpha*. *Chemical Engineering Research and Design* 89(11), 2322-2329.

Costa, R., Moggridge, G. D., Aldridge, D. C. (2012). Improved mussel control through microencapsulated biobullets. In: Rajagopal, S., Jenner, H. A., Venugopalan, V. P. Operational and Environmental Consequences of Large Industrial Cooling Water. Springer, New York. pp. 273-286.

Coutts, A. D., Taylor, M. D. (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.

Coutts, A. D. M., Forrest, B. M. (2005). Evaluation of eradication tools for the clubbed tunicate *Styela clava*. *Cawthron Report No. 1110,* 48 pp.

Coutts, A. D. M., Forrest, B. M. (2007). Development and application of tools for incursion response: Lessons learned from the management of the fouling pest *Didemnum vexillum*. *Journal of Experimental Marine Biology and Ecology* 342(1): 154-162.

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

Crecelius, E. A. (1979). Measurements of oxidants in ozonized seawater and some biological reactions. *Journal of the Fisheries Research Board of Canada* 36(8): 1006-1008.

De Lafontaine, Y., Despatie, S. P., Veilleux, E., Wiley, C. (2009). Onboard ship evaluation of the effectiveness and the potential environmental effects of PERACLEAN Ocean for ballast water treatment in very cold conditions. *Environmental Toxicology* 24(1): 49-65.

Denny, C. M. (2008). Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. *Journal of Marine Science* 65(5): 805–810.

Di Pippo, F., de Tora, F., Di Gregorio, L., Buccolini, M., Capocecera, R., Rossetti, S., Tandoi, V. (2017). Green bio-dispersant removal efficacy estimation for controlling biofilms in cooling towers. *Annals of Microbiology* 67(11): 779-784.

Dunphy, B. J., Wells, R. M. G., Jeffs, A. G. (2005). Polydorid infestation in the flat oyster, *Tiostrea chilensis*: hyposaline treatment for an aquaculture candidate. *Aquaculture International* 13(4): 351-358.

Environmental Protection Authority (2016). Decision APP202796 – Chlorine for biosecurity use. https://www.epa.govt.nz/assets/FileAPI/hsno-ar/APP202796/APP202796-APP202796-Decision-Final-18-8-16.pdf

Fagan, J., Waite, T. D. (1983). Biofouling control with ferrate (VI). *Environmental Science & Technology* 17(2): 123-125.

Ferguson, R. (1999). The effectiveness of Australia's response to the black striped mussel incursion in Darwin, Australia. A report of the marine pest incursion management workshop – 27-28 August 1999, Department of Environment and Heritage, Canberra, Australia, 75 pp.

Forrest, B. M., Blakemore, K. A. (2006). Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. *Aquaculture* 257: 333–345.

Forrest, B., Hopkins, G., Dodgshun, T., Gardner, J. (2007). Efficacy of acetic acid treatments in the management of marine biofouling. *Aquaculture* 262(2-4): 319-332.

Garaventa, F., Corrà, C., Piazza, V., Giacco, E., Greco, G., Pane, L., Faimali, M. (2012). Settlement of the alien mollusc Brachidontes pharaonis in a Mediterranean industrial plant: Bioassays for antifouling treatment optimization and management. *Marine Environmental Research* 76: 90-96.

Golder Associates NZ Ltd (2008). Hull encapsulation as an incursion response tool for marine fouling organisms: a trial on a large vessel. Technical Paper No: 2007. Prepared for MAF Biosecurity New Zealand Project BO_186_2006 Wellington, 19 pp.

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.

Gregg, M., Rigby, G., Hallegraeff, G. M. (2009). Review of two decades of progress in the development of management options for reducing or eradicating phytoplankton, zooplankton and bacteria in ship's ballast water. *Aquatic Invasions* 4(3): 521-565.

Growcott, A., Kluza, D., Georgiades, E. (2016). In-water systems to remove or treat biofouling in vessel sea chests and internal pipework. *MPI Technical Paper No: 2016-16.* 66 pp.

Growcott, A., Kluza, D., Georgiades, E. (2017). Review: In-water systems to reactively manage biofouling in sea chests and internal pipework. *Marine Technology Society Journal* 51(2): 89-104.

Gupta, V. K., Ali, I., Saleh, T. A., Nayak, A., Agarwal, S. (2012). Chemical treatment technologies for waste-water recycling—an overview. *Royal Society of Chemistry - Advances* 2(16): 6380-6388.

Holt, D. A., Ryan, E. (1997). Chlorine dioxide- adult zebra mussel eradication at the Dofasco Steel Mill. Proceedings Seventh International Zebra Mussel and Aquatic Nuisance Species Conference, January 28 to 31, 1997, New Orleans, USA. pp 59-67.

Hose, J. E., Di Fiore, D., Parker, H. S., Sciarrotta, T. (1989). Toxicity of chlorine dioxide to early life stages of marine organisms. *Bulletin of Environmental Contamination and Toxicology* 42(3): 315-319.

Inglis, G., Floerl, O., Woods, C. (2012). Scenarios of vessel biofouling risk and their management. *MAF Research Project RFP11832, Ministry of Agriculture and Forestry, Wellington*, pp. 41-93.

Jenner, H. A., Whitehouse, J. W., Taylor, C. J., Khalanski, M. (1998). Cooling water management in European power stations: biology and control of fouling. *Electricité de France 1*.

Jones, A. C., Gensemer, R. W., Stubblefield, W. A., Van Genderen, E., Dethloff, G. M., Cooper, W. J. (2006). Toxicity of ozonated seawater to marine organisms. *Environmental Toxicology and Chemistry* 25(10): 2683-2691.

Jute, A., Dunphy, B. J. (2016). The potential efficacy and application of freshwater and hypersaline immersion to control the spread of a marine invasive species. *Biological Invasions* 19(4): 1137-1141.

Katayama, K., Ikeda, Z. (1987). Tolerance of fresh water, hot water, and sun-drying by *Didemnum moseleyi*, fouling organisms attached to culture oyster. *Bulletin of the Fisheries Experiment Station, Okayama Prefecture* 2: 104–106.

Kefeli, A. A., Razumovskii, S. D., Zaikov, G. Y. (1971). Interaction of polyethylene with ozone. *Polymer Science* 13(4): 904-911.

Kerrison, P. D., Le, H. N., Twigg, G. C., Smallman, D. R., MacPhee, R., Houston, F. A., Hughes, A. D. (2016). Decontamination treatments to eliminate problem biota from macroalgal tank cultures of *Osmundea pinnatifida*, *Palmaria palmata* and *Ulva lactuca*. *Journal of Applied Phycology* 28(6): 3423-3434.

Klassen, R. D., Roberge, P. R., Porter, J., Pelletier, G., Zwicker, B. (2001). On-board hypochlorite generation for biofouling control. Proceedings of the Corrosion 2001 Conference, Houston, Texas, Nace International. Paper 01480, 11 pp.

Kramer, J. F. (1997). Peracetic acid: A new biocide for industrial water applications. *Materials Performance* 36(8): 42-50.

Lewis, J. A., Dimas, J. (2007). Treatment of biofouling in internal seawater systems-Phase 2 (Report No. DSTO-TR-2081). Defense Science and Technology Organization Victoria (Australia) Maritime Platforms Division. 24 pp.

Liao, J., Kishimoto, K., Yao, M., Mori, Y., Ikai, M., (2012). Effect of ozone on corrosion behavior of mild steel in seawater. *Corrosion Science* 55: 202-212.

Locke, A., Doe, K. G., Fairchild, W. L., Jackman, P. M., Reese, E. J. (2009). Preliminary evaluation of effects of invasive tunicate management with acetic acid and calcium hydroxide on non-target marine organisms in Prince Edward Island, Canada. *Aquatic Invasions* 4(1): 221-236.

Mantelatto, M. C., Pires, L. M., de Oliveira, G. J. G., Creed, J. C. (2015). A test of the efficacy of wrapping to manage the invasive corals *Tubastraea tagusensis* and *T. coccinea*. *Management of Biological Invasions* 6(4): 367-374.

Martin, M.D., Mackie, G.L., Baker, M.A. (1993) Control of the biofouling mollusc, *Dreissena polymorpha* (Bivalvia: Dreissenidae), with sodium hypochlorite and with polyquaternary ammonia and benzothiazole compounds. *Archives of Environmental Contamination and Toxicology* 24(3): 381-388.

Martín-Rodríguez, A. J., Babarro, J. M., Lahoz, F., Sansón, M., Martín, V. S., Norte, M., Fernández, J. J. (2015). From broad-spectrum biocides to quorum sensing disruptors and mussel repellents: Antifouling profile of alkyl triphenylphosphonium salts. *PLoS ONE* 10(4): e0123652.

Masilamoni, G., Jesudoss, K. S., Nandakumar, K., Satapathy, K. K., Azariah, J., Nair, K. V. K. (2002). Lethal and sub-lethal effects of chlorination on green mussel *Perna viridis* in the context of biofouling control in a power plant cooling water system. *Marine Environmental Research* 53(1): 65-76.

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

Matthews, M. A., McMahon, R. F. (1995). Survival of zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) under extreme hypoxia. Texas University at Arlington Department of Biology, Technical Report EL-95-3, 21 pp.

Mayack, L. A., Soracco, R. J., Wilde, E. W., Pope, D. H. (1984). Comparative effectiveness of chlorine and chlorine dioxide biocide regimes for biofouling control. *Water Research* 18(5): 593-599.

McCann, L. D., Holzer, K. K., Davidson, I. C., Ashton, G. V., Chapman, M. D., Ruiz, G. M. (2013). Promoting invasive species control and eradication in the sea: options for managing the tunicate invader *Didemnum vexillum* in Sitka, Alaska. *Marine Pollution Bulletin* 77(1-2): 165-171.

Montresor, L. C., Miranda-Filho, K. C., Paglia, A., Luz, D. M., Araujo, J. M., dos, S. S. M. J., Gerhard, L., Martinez, C. B., Vidigal, T. H. (2013). Short-term toxicity of ammonia, sodium hydroxide and a commercial biocide to golden mussel *Limnoperna fortunei* (Dunker, 1857). *Ecotoxicology and Environmental Safety* 92: 150-154.

Moore, J., Robert, M., Christopher, J. N. (2001). Biocidal applications of concentrated aqueous bromine chloride solutions. U.S. Patent No. 6,322,822. 27.

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

Morrisey, D. J., Depree, C. V., Hickey, C. W., McKenzie, D. S., Middleton, I., Smith, M. D., Stewart, M., Thompson, K. J. (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.

Morrisey, D. J., Woods, C. (2015). In-water cleaning technologies: Review of information. MPI Technical Paper No: 2015/38. Prepared for Ministry for Primary Industries. 54 pp.

Morrisey, D., Inglis, G., Tait, L., Woods, C., Lewis, J., & Georgiades, E. (2015). Procedures for evaluating in-water systems to remove or treat vessel biofouling. MPI Technical Paper No: 2015/39. Prepared for Ministry for Primary Industries. 104 pp.

Neil, K. M., 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 Department of Agriculture, Fisheries and Forestry. Barton, ACT, Australia, 30 pp.

Norton, J.H., Dashorst, M., Lansky, T. M., Mayer, R. J. (1996). An evaluation of some relaxants for use with pearl oysters. *Aquaculture* 144: 39-52.

Nosrati-Ghods, N., Ghadiri, M., Früh, W.-G. (2017). Management and environmental risk study of the physicochemical parameters of ballast water. *Marine Pollution Bulletin* 114(1): 428-438.

Oesch, S., Faller, M. (1997). Environmental effects on materials: The effect of the air pollutants $SO_2$, $NO_2$, NO and $O_3$ on the corrosion of copper, zinc and aluminium. A short literature survey and results of laboratory exposures. *Corrosion Science* 39(9): 1505-1530.

Pannell, A., Coutts, A. D. (2007). Marine pest control tools. Treatment methods used by industry to manage *Didemnum vexillum* in the top of the South Island (Project 764003), Biosecurity New Zealand Report prepared by the Marine Farming Association, Wellington, 46 pp.

Petrille, J. R., Miller, S.D. (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, Feb. 13-17, 2000. 9 pp.

Pimentel, D. (1971). Ecological effects of pesticides on non-target species. Executive Office of the President, Office of Science and Technology, Washington, D.C., USA.

Piola, R. F., Conwell, C. (2010). Vessel biofouling as a vector for the introduction of non-indigenous marine species to New Zealand: Fishing vessels. MAF Biosecurity New Zealand Technical Paper No: 2010/11. 57p.

Piola, R. F., Dunmore, R. A., Forrest, B. M. (2009a). 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. F., Denny, C. M., Forrest, B. M., Taylor, M. D. (2009b). Marine biosecurity: management options and response tools. Invasive species management: A handbook of principles and techniques. Oxford University Press, United Kingdom, pp. 205-231.

Piola, R. F., Grandison, C. (2013). In-water treatment of biofouling in internal systems: Field validation of quaternary ammonium compound (QAC) chemical treatment protocols. *Maritime Platforms Division. Defence Science and Technology Organisation Report No. DSTO-TR-2774*. 47 pp.

Pool, T. K., Luis, S., Olden, J. D. (2013). Assessing lethal dissolved oxygen tolerance for invasive tunicate *Ciona savignyi* in Puget Sound. *Northwest Science* 87(2): 106-11.

Rajagopal, S., Jenner, H. A., Venugopalan, V. P., Khalanski, M. (2012). Biofouling control: alternatives to chlorine. In: S. Rajagopal, H. A. Jenner, V. P. Venugopalan (Eds.), *Operational and Environmental Consequences of Large Industrial Cooling Water Systems.* Boston, MA, Springer US. pp. 227-271.

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

Rajagopal, S., Van Der Velde, G., Jenner, H. A. (1997). Shell valve movement response of dark false mussel, *Mytilopsis leucophaeta*, to chlorination. *Water Research* 31(12): 3187-3190.

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

Rajagopal, S., Venugopalan, V. P., Nair, K. V. K., Azariah, J. (1995). 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.

Roche, R. C., Monnington, J. M., Newstead, R. G., Sambrook, K., Griffith, K., Holt, R. H. F., Jenkins, S. R. (2015). Recreational vessels as a vector for marine non-natives: developing biosecurity measures and managing risk through an in-water encapsulation system. *Hydrobiologia* 750(1): 187-199.

Rolheiser, K. C., Dunham, A., Switzer, S. E., Pearce, C. M., Therriault, T. W. (2012). Assessment of chemical treatments for controlling *Didemnum vexillum*, other biofouling, and predatory sea stars in Pacific oyster aquaculture. *Aquaculture* 364-365: 53-60.

Saia, F., Domingues, M., Pellizari, V., & Vazoller, R. (2010). Occurrence of methanogenic Archaea in highly polluted sediments of tropical Santos–São Vicente Estuary (São Paulo, Brazil). *Current Microbiology* 60(1): 66.

Sharma, V. K. (2002). Potassium ferrate VI : An environmentally friendly oxidant. *Advances in Environmental Research* 6: 143-156.

Statistics New Zealand (2016) National Environmental indicators Marine-Pests. http://archive.stats.govt.nz/browse_for_stats/environment/environmental-reporting-series/environmental-indicators/Home/Marine/marine-pests/marine-pests-archived-27-10-16.aspx

Stewart, P., Rayner, J., Roe, F., Rees, W. (2001). Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. *Journal of Applied Microbiology* 91: 525-532.

TenEyck, M. (2009). Sodium hydroxide (NaOH). Great Ships Initiative Bench-Scale Test Findings. *Technical Report—Public GSI/BS/5*.

Thomas, K. V., Brooks, S. (2010). The environmental fate and effects of antifouling paint biocides. *Biofouling* 26(1): 73-88.

Thompson, I. S., Richardson, C. A. (1993). The response of the common cockle, *Cerastoderma edule*, to simulated chlorination procedures. *Biofouling* 7(4): 299-312.

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

Ullmann's Encyclopedia of Industrial Chemistry. Chlorine Oxides and Chlorine Oxygen Acids. (Uhlmann). In *Ullmann's Encyclopedia of Industrial Chemistry*.

Understanding ballast water management - Guidance for shipowners and operators. (2015). Lloyd's Register Marine. Retrieved from www.lr.org/bwm

Verween, A., Vincx, M., Degraer, S. (2009). Comparative toxicity of chlorine and peracetic acid in the biofouling control of *Mytilopsis leucophaeata* and *Dreissena polymorpha* embryos (Mollusca, Bivalvia). *International Biodeterioration & Biodegradation* 63(4): 523-528.

Viera, M. R., Guiamet, P. S., De Mele, M. F. L., Videla, H. A. (1999). Use of dissolved ozone for controlling planktonic and sessile bacteria in industrial cooling systems. *International Biodeterioration & Biodegradation* 44(4): 201-207.

Walker, J. T., Rogers, J., Keevil, C. W. (2009). An investigation of the efficacy of a bromine containing biocide on an aquatic consortium of planktonic and biofilm micro-organisms including *Legionella pneumophila*. *Biofouling* 8(1): 47-54.

Werschkun, B., Banerji, S., Basurko, O. C., David, M., Fuhr, F., Gollasch, S., Grummt, T., Haarich, M., Jha, A. N., Kacan, S., Kehrer, A., Linders, J., Mesbahi, E., Pughiuc, D., Richardson, S. D., Schwarz-Schulz, B., Shah, A., Theobald, N., von Gunten, U., Wieck, S., Höfer, T. (2014). Emerging risks from ballast water treatment: The run-up to the International Ballast Water Management Convention. *Chemosphere* 112: 256-266.

Williams, S. L., Schroeder, S. L. (2004). Eradication of the invasive seaweed *Caulerpa taxifolia* by chlorine bleach. *Marine Ecology Progress Series* 272: 69-76.

Wiltshire, K. (2007). Ecophysiological tolerances of the Pacific oyster, *Crassostrea gigas*, with regard to the potential spread of populations in South australanin waters. SARDI Aquatic Sciences Publication Number F2007/000499-1. SARDI Report Series Number 222, 29 pp.

World Health Organization (2018). Alternative drinking-water disinfectants: bromine, iodine and silver. Geneva: World Health Organization. *Licence: CC BY-NC-SA 3.0 IGO.*

Wotton, D. M., O'Brien, C., Stuart, M. D., Fergus, D. J. (2004). Eradication success down under: heat treatment of a sunken trawler to kill the invasive seaweed *Undaria pinnatifida*. *Marine Pollution Bulletin* 49(9): 844-849.

Wright, D. A., Dawson, R., Mackey, T. P., Cutler, H. G., Cutler, S. J. (2004) Some shipboard trials of ballast water treatment systems in the United States. In: Matheickal, J. T., Raaymakers, S. (eds), Second International Symposium on Ballast Water Treatment. International Maritime Organisation, London, UK, pp 243-257.

Yates, B. J., Zboril, R., Sharma, V. K. (2014). Engineering aspects of ferrate in water and wastewater treatment – a review. *Journal of Environmental Science and Health, Part A* 49(14): 1603-1614.

Yu, L., Guo, Q., Hao, J., & Jiang, W. (2000). Recovery of acetic acid from dilute wastewater by means of bipolar membrane electrodialysis. *Desalination* 129(3): 283-288.

Zhang, C., Cui, F., Zeng, G. M., Jiang, M., Yang, Z. Z., Yu, Z. G., Zhu, M. Y., Shen, L. Q. (2015). Quaternary ammonium compounds (QACs): a review on occurrence, fate and toxicity in the environment. *Science of the Total Environment* 518-519: 352-362.