

Research Article

An assessment of the efficacy of chemical descalers for managing non-indigenous marine species within vessel internal seawater systems and niche areas

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Abstract

This study assessed the efficacy of commercially available descalers and factors that influence their efficacy as tools for marine biosecurity management. Laboratory experiments found calcium carbonate (CaCO₃) degradation varied up to 29% (from 111 to 143 g/l) amongst seven products tested. Increasing the concentration of hydrochloric, phosphoric and acid-surfactant descalers from 25 to 75% did not increase the rate or total degradation of the mussel, *Mytilus planulatus*. Warming descaling solutions (from 11 to 26°C) significantly increased the rate of mussel mortality, decay and total degradation in all treatments. Circulating treatments increased mussel mortality and decay rate in hydrochloric and acid-surfactant descalers, but had no detectable effect on total degradation after 24h. Hydrochloric acid based descalers (Rydlyme[®], 3H[®] and Dynamic Descaler[®]) were more effective than phosphoric acid (Barnacle Buster[®]) and acid-surfactant (Triple 7 EnviroScale Plus[®]) treatments. Organic material was largely resistant to degradation under all treatments. The implications for descalers as marine biosecurity tools are discussed

Key words: non-indigenous species, mussels, biofouling, calcium carbonate

Introduction

Marine non-indigenous species (NIS) continue to be translocated by vessels outside their natural biogeographical range (Chapman et al. 2013). Establishment and proliferation of an NIS can disrupt and/or damage local ecology (Ruiz et al. 1997), industry (Dürr and Watson 2010), human health (Drake et al. 2007), social and cultural values (Pejchar and Mooney 2009). Vessels can transport NIS via multiple mechanisms, however recent research suggests that biofouling is the greatest current threat to new introductions in some locations (Thresher et al. 1999; Gollasch 2002; Hewitt and Campbell 2008; Williams et al. 2013). Biofouling organisms can colonise and grow on any unprotected, wetted vessel surface. Submerged hulls, niche areas (e.g., sea chests, propeller rope guards, bow thruster tunnels) and internal seawater systems (piping and associated components: valves, pumps, strainers and joints) can all accumulate biofouling and NIS over time.

Internal seawater systems and niche areas can pose particular biosecurity risks since they can provide refuges for NIS, where favourable environmental conditions can facilitate a high survival rate during vessel passage. Biofouling in these areas may benefit from a continuous flow of oxygen, nutrients and elevated water temperatures (Coutts and Dodgshun 2007), inaccessibility of surfaces for maintenance and biofouling protection (Grandison et al. 2012), lack of predators and reduced inter-specific competition for space (Satpathy 2010), flushing of metabolic waste (Lewis et al. 1998) and protection from severe hydrodynamic shear forces (Coutts et al. 2010). Niche areas have frequently been observed to possess a greater abundance and diversity of biofouling organisms than the general hull (Rainer 1995; Coutts and Taylor 2004; Clarke-Murray et al. 2011; Lacoursière-roussel et al. 2012), with recent studies indicating that sea chests pose a significant threat for new NIS incursions if not effectively managed (Coutts et al. 2003; Coutts and Dodgshun 2007; Frey

et al. 2014). Biofouling in vessel internal seawater systems is common (Gust and Coutts pers. obs.) and has been reported in the literature (Jones and Little 1990; Booth and Wells 2012); however its relative importance as a mechanism for NIS transfers and effective remediation strategies remain poorly studied.

A range of management strategies exist that aim to inhibit biofouling assemblages from developing and surviving within internal seawater systems and niche areas. These include toxic materials (copper or nickel piping), marine growth prevention systems (MGPS) that release biocides such as chlorine (e.g., *Chloropac*[®]) or copper ions (e.g., *Cathelco*[®]), anti-fouling coatings and osmotic shock treatments (Taylor and Rigby 2002; Grandison et al. 2012). These management systems show variable efficacy and typically require maintenance to avoid deterioration and biofouling accumulation over time. The most pragmatic and effective strategy for preventing new NIS incursions via vessel biofouling (particularly for vessels with long docking cycles) is to use effective, appropriate anti-fouling systems, and ensure their maintenance via regular in-water inspection and biofouling removal (Lewis and Coutts 2010).

NIS may be removed from seawater systems by manual cleaning, however this is highly labour intensive (Taylor and Rigby 2002) and some areas may remain inaccessible. Alternatively, treatment with chemical solutions enables NIS to be eliminated rapidly from remote sites without the need to dismantle large sections of piping or access enclosed niches such as rope guards. A large range of chemical treatments may be used to kill and/or remove biofouling. Chemical treatments can be broadly categorised into oxidising biocides (e.g., sodium hypochlorite), non-oxidising biocides (e.g., benzalkonium chloride) and chemical cleaners (descalers). Non-chemical treatments such as thermal shock (Leach 2011; Piola and Hopkins 2012) and more “eco-friendly” chemicals such as dilute acetic acid (Piola et al. 2010) have also received attention as marine biosecurity management tools. The efficacy of a range of chemical solutions previously tested against mussels is shown in Table 1.

Regardless of the treatment options adopted, for biosecurity applications it remains imperative that the chemical solution administered provides certainty of success (i.e. 100% mortality and/or elimination of all target organisms). It is also particularly important for industry that treatments meet this demand efficiently as costs escalate rapidly as vessel schedules are delayed. Depending on the type of NIS, oxidising and non-oxidising biocides may not provide the necessary assurance of success within practical time

frames. For instance, in the presence of biocides some taxa such as mussels and oysters can protect internal tissues by closing their shell valves (Rajagopal et al. 1997; Neil and Stafford 2005) and similarly serpulids can close their operculum (Forrest et al. 2007) to limit exposure to lethal treatments. In Australia, increasingly stringent regulations requiring that vessels be completely free of secondary and tertiary biofouling (e.g., invertebrates) post-treatment (Booth and Wells 2012) and industry requirements for timely, assured decontamination has driven widespread use of chemical descalers for marine biosecurity applications.

Descalers are proprietary chemical mixtures commonly used in the maritime industry for removing metal carbonates (scale) from cooling systems to improve mechanical performance and heat exchange. Most descalers contain an acid such as hydrochloric, phosphoric, sulfamic, citric and/or lactic. Additional constituents such as corrosion inhibitors, surfactants, colour indicators, organic acids and salts may also be disclosed by the manufacturer.

Descalers remove scale via a chemical reaction between the treatment acid(s) and alkaline scale, producing carbon dioxide and a salt. Many NIS construct calcium carbonate (CaCO_3) shells (e.g., mussels, oysters and barnacles) and casings (e.g., polychaetes) from calcite and/or aragonite crystals. This calcareous biofouling reacts rapidly when exposed to acidic descaling treatments, effectively “dissolving” the biofouling and causing mortality. The chemical degradation of biofouling forms the mechanism and rationale for descaler use in marine biosecurity applications. Following descaler treatments, examination of cooling systems often reveals completely clean piping (Gust, pers. obs.), suggesting that invertebrate organics may also be degraded, presumably by acid-hydrolysis.

Despite significant uncertainties surrounding the relative efficacy of products and treatment regimes, contemporary application of descalers for treating NIS in seawater systems and niche areas has involved very large volumes (cubic metres) of solution at high cost (tens to hundreds of thousands of dollars). Surprisingly, the influence of fundamental variables such as choice of descaler type, treatment time, concentration, temperature, circulation and the efficacy against a variety of target taxa remain poorly understood and largely untested.

To assess these uncertainties, inform regulators and assist industry best practice, the present study reports on laboratory experiments designed to test the efficacy of descalers as tools for marine biosecurity. Three sequential phases of experimentation were carried out. Phase one assessed the capacity for seven

Table 1. Summary of research into the efficacy of various oxidising and non-oxidising chemical treatments against marine and freshwater mussels.¹

Species	Treatment	Efficacy	Authors
<i>Dreissena polymorpha</i> (Pallas, 1771)	Review of oxidizing, non-oxidising and metallic molluscicides	Various, 100% mortality in hours-weeks depending on chemical type and dose	McMahon et al. (1994)
<i>D. polymorpha</i>	Proprietary formulation <i>Peraclean</i> [®] - peracetic acid, 150ppm for 5 days	ineffective	de Lafontaine et al. (2009)
<i>D. polymorpha</i>	<i>BioBullets</i> [®] , encapsulated potassium chloride, 24h exposure	60% mortality	Aldridge et al. (2006)
<i>D. polymorpha</i>	Three treatments (pH levels 2, 3, 4) of aqueous phosphoric acid, for 96h	Treatment mortality for adult mussels recorded at 69.9%, 100% and 52.4% respectively	Claudi et al. (2012)
<i>Dreissena rostriformis bugensis</i> (Andrusov, 1897)	Proprietary formulation <i>EarthTec</i> [®] - cupric copper ion Cu ²⁺ , 83ppm for 30h	50% mortality	Watters et al. (2013)
<i>Perna viridis</i> (Linnaeus, 1758)	9.1mg/l chlorine, 94h for 3–4 cm mussels, 114h for 8–9cm mussels	100% mortality	Masilamoni et al. (2002)
<i>Perna viridis</i> <i>Perna perna</i> (Linnaeus, 1758) <i>Brachidontes striatulus</i> (Hanley, 1843) <i>Brachidontes variabilis</i> (Krauss, 1848) <i>Modiolus philippinarum</i> (Hanley, 1843)	10-15mg ⁻¹ chlorine for 48h	100% mortality for all species (variable sizes), authors noted that tolerance increased with size	Rajagopal et al. (2003)
<i>Mytilus galloprovincialis</i> small (10–30mm) large (55–80mm)	10min heat shock, 40°C for small, 42.5°C for large mussels	100% mortality	Piola and Hopkins (2012)
<i>M. galloprovincialis</i>	1% <i>Quatson</i> [®] 1% <i>Conquest</i> [®] (both benzalkonium chloride)	100% mortality after 14hr immersions (detected after 24h for <i>Quatson</i> [®] , 48h for <i>Conquest</i> [®])	Lewis and Dimas (2007)
<i>M. galloprovincialis</i> 25–65mm	10% vinegar for 12h	75% mortality	Lewis and Dimas (2007)

¹Mussels are common vessel biofouling taxa, known for their strong invasiveness potential and resilience to environmental stresses. This table does not provide an exhaustive list of molluscicides, but provides examples of the observed biocidal action of different treatments (oxidising, non-oxidising and more eco-friendly).

Table 2. Known characteristics of the seven descaling solutions assessed in this study (plus HCl control treatment used in phase two). The proprietary nature of the products precludes detailed description of their chemical composition.

Product	Abbreviation	Aqueous constituents and concentrations as stated in MSDS	Appearance
<i>triple7 EnviroScale Plus</i> [®]	<i>t7EP</i>	Citric acid (30–60%) Lactic acid (30–60%) Surfactants (<5%)	Transparent, viscous
<i>Barnacle Buster concentrate</i> [®]	<i>BB</i>	Phosphoric acid (40–85%)	Pale blue, viscous
<i>Rydlyme</i> [®]	<i>Rydlyme</i>	Hydrogen chloride (<10%)	Black
<i>Rydlyme Marine</i> [®]	<i>RdM</i>	Hydrogen chloride (<10%)	Black
<i>Dynamic Descaler</i> [®]	<i>DD</i>	Hydrogen chloride (<10%)	Pale orange
<i>3H Descaler</i> [®]	<i>3H</i>	Hydrogen chloride (5–9%)	Pale yellow
<i>3H Marine</i> [®]	<i>3HM</i>	Hydrogen chloride (5–9%)	Pale yellow
<i>Diggers</i> [®] Hydrochloric acid	HCl-control	Hydrogen chloride (approx. 23% w/w)	Transparent

commercially available descalers to react CaCO_3 by experimentally determining CaCO_3 degradation. Phase two analysed the degradation of the mussel *M. galloprovincialis* through time under different treatment concentrations and descaler types. Phase three tested the effect of treatment temperature and circulation on the degradation and mortality of *M. galloprovincialis* for three descaler types.

Methods

General

Descalers

Seven commercially available descaling solutions were examined in this study. These included five hydrochloric acid (HCl) based descalers: *Rydlyme Marine*[®] (*RdM*), *Rydlyme*[®], *3H*[®], *3H Marine*[®] (*3HM*), *Dynamic Descaler*[®] (*DD*), the phosphoric acid descaler *Barnacle Buster*[®] concentrate (*BB*), and the acid-surfactant descaler *triple 7 EnviroScale Plus*[®] (*t7EP*) containing citric and lactic acids. In all experiments, *BB* and *t7EP* concentrates were first diluted at 1:2 with freshwater based on manufactures recommendations for treating particularly heavy biofouling. Table 2 provides a summary of the known properties of these descalers.

Test organisms and study location

Blue mussels were used as test subjects in phase two and three trials. These organisms are widely referred to as *M. galloprovincialis*, but consist of a hybridised species complex that Astorga et al. 2015 recently suggested should be renamed *M. planulatus* in the southern hemisphere. This organism is referred to hereafter as *Mytilus* and was selected since it is a common biofouling mussel of vessel niche areas (Coutts and Dodgshun 2007; Lee and Chown 2007) that has been widely tested in chemical treatment experiments. *Mytilus* were collected from a mid-intertidal rocky reef at Blackmans Bay south-east Tasmania (43°00'27.5"S, 147°19'42.7"E) and from pontoons at the Royal Yacht Club of Tasmania, Hobart (42°53'53.3"S, 147°20'2"E) for phases two and three, respectively.

Mussels of comparable size (approximately 40–60mm shell length) and shape were selected to reduce variability associated with surface-area-to-volume ratios (SA:V), shell thickness and condition of the periostracum (the organic layer comprised of sclerotinised protein exterior to the calcified shell; Harper 1997). Any *Mytilus* with obvious shell abnormalities (e.g. bulges caused by pea crab parasitism; Edgar 2008) were not trialled to reduce variability in shell weight between specimens.

Mussels were held in an insulated aquarium (750×450×400mm) fitted with an aerator. Saltwater was replaced twice weekly. Experiments were conducted at Biofouling Solutions Pty. Ltd.'s laboratories at Kingston, south-eastern Tasmania.

Phase one: descaler CaCO_3 degradation

Design and technique

An experimental approach was required to identify descaler CaCO_3 degradation since the proprietary nature of descalers prevented theoretical appraisals. Seven descalers were tested independently by addition of excess laboratory grade CaCO_3 (*Chem-supply*[®]) to four volumes of solution: 25, 50, 75 and 100ml with three replicates of each treatment ($n=12$). Aliquots of homogenised descaler were contained in one litre glass beakers and diluted at 1:1 ratio with freshwater to prevent excessive foaming. Approximately 5, 8, 12 and 15g of CaCO_3 for volumes 25, 50, 75 and 100ml respectively, were weighed to 0.0001g (using an *Ohaus Explorer*[®] electronic scale) before addition to treatments. Experiments were carried out at ambient temperature (approx. 10°C) and were given 24hours to reach equilibrium, confirmed by the cessation of bubbling. Remaining CaCO_3 was washed into a 250 μm sieve, transferred to a petri-dish and air-dried for at least 72h. Once dry each replicate was reweighed to the nearest 0.0001g.

Descaler CaCO_3 extrapolations

Linear regression coefficients from phase one results were used to predict the expected CaCO_3 degradation in one litre of each descaler. These data were then converted to whole *Mytilus* shells to provide a more intuitive estimate of descaler efficacy by using a relationship between *Mytilus* length and shell weight for organisms collected from Blackmans Bay. This relationship was determined in a pilot study and is presented here in Figure 1. Estimates of the number of mussels compromised through complete shell dissolution were made based on a medium sized *Mytilus* of 40mm with a corresponding shell weight of approximately 2.5g. It is noted that this estimate ignores the organic component of shells and is thus likely to be slightly conservative.

Phase two: the effect of concentration and descaler type

Design and materials

This experiment was conducted as a two-way ANOVA with the fixed factors, concentration and descaler type. Five descalers (*RdM*, *DD*, *BB*, *3HM*,

t7EP) and a dilute hydrochloric acid control (HCl-control) solution were tested at four levels of concentration 12.5, 25, 50, and 75%, with five replicates ($n=120$). Concentrations were selected on the basis of pilot trials which indicated that *Mytilus* degraded in 12.5% treatments, but not in 6.25%, and also to encompass levels previously applied during vessel treatments (Coutts and Gust, pers. obs.). All treatments were 0.4 litres. This volume provided a conservative approximation of the minimum descaler volume required to react the mean *Mytilus* CaCO_3 shell mass (5.1g) at the lowest concentration (12.5%) and was informed by applying descaler CaCO_3 regression analysis (from phase one) and the relationship between *Mytilus* length and shell weight for organisms from Blackmans Bay (Figure 1). Mussels were weighed systematically at time intervals 0, 2, 4, 8, 12, 24 and 48h. Intervals were selected based on the duration for which vessels are normally treated for biosecurity applications (Coutts and Gust, pers. obs.).

Aliquots of each descaler were diluted with freshwater (as recommended by the manufactures) to the desired concentrations and contained in one litre glass beakers. HCl-control treatments were made from *Diggers*[®] hydrochloric acid. A concentration of approximately 23% w/w hydrogen chloride was calculated based on pH; therefore a 1:2 dilution was suitable as this placed the concentration in a comparable range with *DD*, *RdM* and *3HM* (5–9% undiluted). Temperature was controlled by containing the beakers in a large polypropylene water bath (1030×720×320mm) with water pumped through a heater/chiller unit set at $11^\circ\text{C} \pm 2^\circ\text{C}$. Pilot studies showed that in descaling treatments mussels float post-mortality; this was identified as a source of error as the entire surface of the organism was no longer in contact with descaler. To contain test organisms within the solution at all times, stoppers were utilised; these consisted of a PVC mesh (0.5mm) circle affixed to an acrylic rod (0.8mm diameter) at a length equivalent to half the depth of the solution.

Laboratory technique

Each descaler was tested over an independent 48h period, thus 25 trials (four concentration levels + 0% mortality control with five replicates) were carried out concurrently. Thirty mussels were randomly selected and removed of calcareous epibionts (barnacles, serpulids and spirorbids) to prevent variability caused by their dissolution; byssus threads were left attached to observe if descalers could degrade them. Mussels were weighed to the nearest

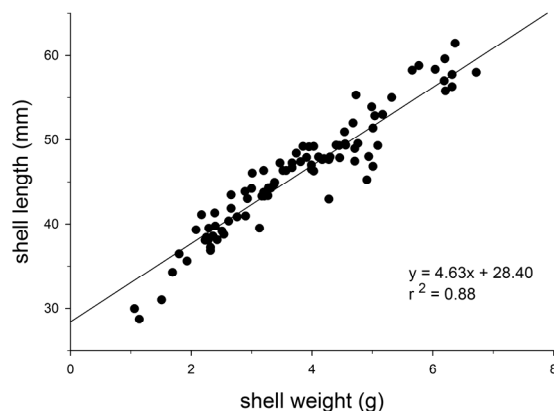


Figure 1. A relationship between *Mytilus* shell length and shell weight for 90 individuals collected at low tide from Blackmans Bay.

0.01g before addition to a treatment. At time intervals 0, 2, 4, 8, 12, 24 and 48h test subjects were removed from treatment solutions and placed on absorptive paper towel to remove surface water for one minute before weighing. During this time individual mussels were oriented on dorsal, ventral and radial edges to ensure even drying. Observations on the extent and region of shell dissolution and the condition of the periostracum, byssus and viscera were recorded. Organisms were returned to their respective treatments after two minutes.

Phase three: the effect of temperature and circulation

Design and materials

This experiment was analysed using a nested model with the fixed factors, treatment and circulating system. *Mytilus* degradation and mortality was assessed for three descaler types: HCl (*3HM*), acid-surfactant (*t7EP*) and phosphoric acid (*BB*) in three treatments: heat ($26 \pm 2^\circ\text{C}$, static), circulation ($11 \pm 2^\circ\text{C}$, $\sim 0.25\text{ms}^{-1}$) and a control ($11 \pm 2^\circ\text{C}$, static). Three independent replicates were used per treatment, each containing five *Mytilus* ($n=45$ per descaler). Treatment concentration was fixed at 25% based on phase two results which showed comparably high efficacy at this concentration. A lower concentration was also considered less likely to interact with the main effects. The 48h interval examined in phase two was omitted as little degradation occurred post-24h.

Experiments were conducted in three purpose-built circulating systems constructed from 100mm diameter polyvinyl chloride (PVC) plumbing pipe and were approximately 600×385mm. Circulation was achieved with *Rule*[®] 360GPH submersible 12 volt bilge pumps fixed to the base of each system, with

the outlet parallel to the pipe walls. Pumps were initially pilot tested with acidic descaling treatments over a 24 hour period to ensure compatibility. The three systems were contained side-by-side in the same large water bath (with heat/chiller unit) used in phase two. The systems held eight litres of treatment solution. Test subjects were contained in identical nylon mesh bags (2–5mm mesh) with a cable tie used to contain organisms at the bottom of bags. A peg was screwed into the top of the system to secure and stabilise the bags during treatment.

Laboratory technique

Mussels were weighed and examined for the effects of treatment as described in phase two. Mortality was assessed by examining organisms for valve gape and physically stimulating exposed mantle tissue and/or gently prying shell valves to test for function as described by LeBlanc et al. (2005). It was assumed that specimens had died once shell valves failed to retract or did so very slowly, and/or when mantle tissue became unresponsive to stimuli. Mussels remained out of treatment for approximately eight minutes.

Data analysis

SigmaPlot Version 12.5 was used to construct linear regressions (phase one) and line and scatterplots (phases two and three). To allow comparison of *Mytilus* degradation rates (phases two and three) over the first eight hours of treatment when rapid decay and high variability obscured simple observations, the slope of the curve (m) was calculated. Analysis of variance was conducted using R (*R Core Team* 2014) to compare *Mytilus* degradation between treatments. Significance was fixed at a probability $P=0.05$. Following exploratory tests for homoscedasticity in phase two, data were power transformed (2.35, 3.0 and 5.3 for analyses at 12, 24 and 48h, respectively) to satisfy assumptions of normality.

Treatments were compared by conducting independent analysis at 12, 24 and 48h (phase two) and 12 and 24h (phase three). Multiple comparisons (phases two and three) were conducted using Tukey's Honest Significance Difference (HSD) Test to control the Type 1 error rate. In phase three, where the overall ANOVA indicated $p>0.25$ for the nested component (treatment system), this term was removed to increase the power to detect a difference between main treatment effects (Winer 1971). Boxplots were constructed using the *multcompView* package (Graves et al. 2012) in R to compare descaler types in phase two.

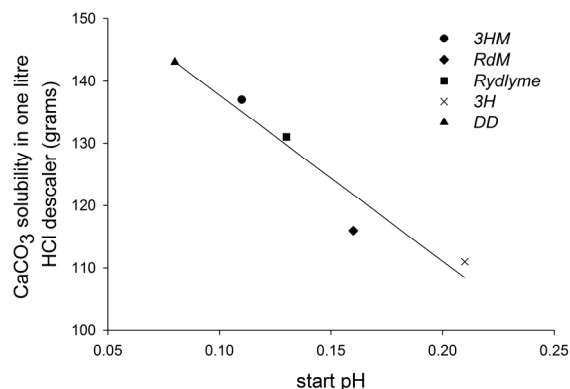


Figure 2. The relationship between CaCO_3 degradation and descaler starting pH for HCl based descalers. Descaler CaCO_3 degradation per litre is plotted against descaler starting pH at a 1:1 dilution with freshwater.

Results

Phase one: descaler CaCO_3 degradation

A summary of CaCO_3 degradation in seven descaling solutions is presented in Table 3. In each case r^2 values of 0.99 indicate high precision in the degradation estimates under experimental conditions. *DD* reacted the most CaCO_3 (estimated at 143g/l), 29% more than *3H* which reacted the least (estimated at 111g/l). Based on CaCO_3 degradation in one litre of descaler the number of medium size (40mm) *Mytilus* that could be compromised through the complete dissolution of the calcareous shell was 44–57 across products. pH values indicate that solutions were highly acidic at the beginning of trials and failed to neutralise at the end of the experiment. Lower starting pH for the HCl descalers was correlated with higher capacity to react CaCO_3 (Figure 2).

Phase two: the effect of concentration and descaler type

All descaler treatments caused mortality in *Mytilus* with partial or complete dissolution of the calcareous shell. The maximum mean weight reduction across treatments was approximately 75%, with the remaining mass comprising periostracum, mantle, viscera and byssus threads. *Mytilus* tissue was resistant to degradation and remained largely intact under all treatments at 48h. Mussel structural integrity was compromised initially through failure of the mechanical action of the hinge and/or ligament. This was indicated by the absence of ligament response to stimuli, and/or the hinge (anterior region) becoming

Table 3. Summary of phase one data showing linear regression equations for the seven descalers tested and respective estimates of efficacy. pH values are the median of three replicates.

Product	Equation	r^2	Predicted CaCO ₃ mass (g) reacted with one litre descaler	Approx. number of 40mm <i>Mytilus</i> (CaCO ₃) digested with 1 litre of descaler	Start pH	Finish pH
<i>DD</i>	$y = 0.14 + 0.09$	0.99	143	57	0.08	4.60
<i>3HM</i>	$y = 0.14 + 0.07$	0.99	137	55	0.11	5.10
<i>BB</i>	$y = 0.13 - 0.16$	0.99	135	54	0.90	2.10
<i>Rydllyme</i>	$y = 0.13 + 0.04$	0.99	131	52	0.13	5.10
<i>t7EP</i>	$y = 0.13 + 0.56$	0.99	131	52	1.70	3.70
<i>RdM</i>	$y = 0.12 + 0.05$	0.99	116	46	0.16	4.90
<i>3H</i>	$y = 0.11 + 0.05$	0.99	111	44	0.21	5.40

dissociated from the shell valves. Under all treatments, *Mytilus* shell dissolution was uneven, however patches of complete shell loss were commonly observed first at the anterior, umbos region particularly in close proximity to the hinge. In *t7EP* trials *Mytilus* accumulated a white precipitate beneath the periostracum and/or remnant shell, viscera and shell were regularly dissociated and the periostracum was softer and more easily damaged relative to other descalers.

Mytilus degradation

Mytilus in the three HCl descalers (*RdM*, *DD*, and *3HM*) showed similar patterns of degradation across the four concentrations (Figure 3, plots A, B and C, respectively). Decay rates for 25, 50 and 75% treatments were comparable in each case; weight loss showed a pattern of exponential decay from 0–8h, small additional declines from 12–24h before reaching a plateau thereafter. By 12h a mean 92% of the total weight loss was recorded for HCl descalers at concentrations $\geq 25\%$. *Mytilus* weight reduced more slowly in 12.5% treatments but still converged on a similar weight to all higher concentrations by 48h. HCl-control trials showed evidence of an association between increasing levels of concentration and more rapid mussel decay, an effect not apparent for the HCl descalers (Figure 4).

Mytilus degraded more slowly in *t7EP* trials (Figure 5). Similarly to the HCl descalers, mussels exposed to higher concentrations of *t7EP* (25, 50, 75%) showed comparable rates of degradation. A plateau in decay for $\geq 25\%$ treatments was observed

at 24h, at which point 99% of total degradation had occurred. Mussels in 12.5% *t7EP* degraded substantially more slowly than higher concentrations over the first eight hours of treatment and had yet to plateau at 24h (approximately 78% of total weight loss).

BB trials showed particularly high variability between replicates from 0–12h which made it difficult to discern clear effects of concentration (Figure 6). Faster initial decay rate is speculated for *Mytilus* in 75% *BB*. In all *BB* treatments, the rate of *Mytilus* decay had slowed by 24h, at which point approximately 92% total weight loss was recorded.

Analysis of Mytilus mass removed at 12, 24 and 48h

Descaler type and concentration had a significant effect on the degradation of *Mytilus* at 12 (ANOVA : $F_{(5,96)} = 22.62, P = <0.000$) and 24h (ANOVA : $F_{(5,96)} = 20.56, P = <0.000$) with a significant interaction effect at 48h (ANOVA : $F_{(15,96)} = 2.14, P = <0.014$). The effect of descaler type at 12 and 24h is shown in Figure 7. HCl descalers and the HCl-control showed no significant differences in mean *Mytilus* mass removed. *BB* and *t7EP* removed significantly less mass than the HCl descalers at both time intervals. Table 4 shows significant descaler comparisons at 48h responsible for the interaction effect. Large differences in the magnitude of mean mussel weight were apparent between treatments. For example, at 12.5% HCl descalers and *BB* all removed significantly more mass than *t7EP*. Significant pairwise comparisons for the effect of concentration at intervals 12, 24 and 48h are shown in Table 5. Only the lowest level of concentration (12.5%)

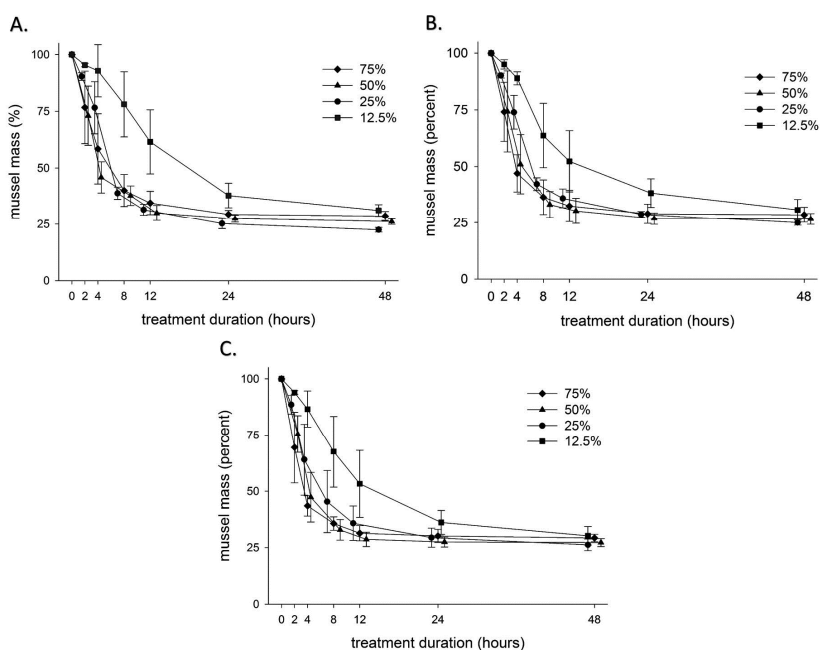


Figure 3. *Mytilus* degradation in 12.5, 25, 50 and 75% *RdM* (A), *DD* (B) and *3HM* (C) HCl descaler treatments. Each point is the mean of five replicate mussels and plotted with ± 2 SE. Rate of decay after eight hours exposure: *RdM* [75%] $m = -7.49$, [50%] $m = -7.78$, [25%] $m = -7.64$, [12.5%] $m = -2.74$, *DD* [75%] $m = -7.94$, [50%] $m = -8.36$, [25%] $m = -7.22$, [12.5%] $m = -4.54$, *3HM* [75%] $m = -8.04$, [50%] $m = -8.39$, [25%] $m = -6.83$, [12.5%] $m = -4.05$.

Table 4. Descaler comparisons identifying the product/concentration combinations that had caused significantly more mussel weight loss at 48h.

Significant descaler comparison	P*
[75%] <i>RdM</i> > <i>t7EP</i>	0.037
[75%] <i>DD</i> > <i>t7EP</i>	0.022
[25%] <i>RdM</i> > <i>BB</i>	<0.000
[25%] <i>DD</i> > <i>BB</i>	0.016
[12.5%] <i>RdM</i> > <i>t7EP</i>	0.015
[12.5%] <i>DD</i> > <i>t7EP</i>	0.005
[12.5%] <i>3HM</i> > <i>t7EP</i>	0.002
[12.5%] <i>BB</i> > <i>t7EP</i>	0.016

removed significantly less *Mytilus* mass under certain treatments. No evidence was observed to suggest that higher concentrations (50 or 75%) were more effective than 25% at reducing *Mytilus* weight. Across all treatments at 24 and 48h, the highest descaler concentration (75%) did not show a statistically significant advantage for degrading *Mytilus* over the lowest concentration (12.5%).

Phase three: the effect of circulation and temperature

Mytilus tissue remained intact at 24h with no obvious signs of degradation. Dissociation of shell and periostracum from the viscera was observed, particularly in circulation and heat trials for *BB* and *t7EP* treatments. The accumulation of a white precipitate under the periostracum and shell of mussels exposed to *t7EP* was observed in all three treatments.

Mytilus degradation and mortality effects 3HM treatments

Heat and circulation treatments increased the rate of *Mytilus* degradation relative to the control with heat treatments showing the fastest decay rate over the first eight hours (Figure 8, plot A). A significant treatment effect was observed both at 12 (ANOVA : $F_{(2,42)} = 11.72$, $P = <0.000$) and 24h (ANOVA: $F_{(2,42)} = 8.56$, $P = 0.001$). Heat removed significantly more mussel mass than control and circulation treatments at 12 and 24h; circulation was not statistically different from the control at either time interval (Table 6). A significant difference was not detected between replicate treatment systems. Rapid *Mytilus* mortality was observed; all specimens were dead at eight hours in heat and circulation trials, and at 12h for the control (Figure 8, plot A).

t7EP treatments

Mytilus degraded more quickly under heat and circulation treatments with the fastest weight loss observed in heat treatments over the first eight hours (Figure 8, plot B). A significant treatment effect was observed at 12 (ANOVA: $F_{(2,36)} = 32.17, P = <0.000$) and 24h (ANOVA: $F_{(2,36)} = 18.22, P = <0.000$). Heat and circulation both reduced *Mytilus* weight significantly more than the control at 12 and 24h, with heat also showing greater efficacy than circulation at 12h (Table 6). A significant difference was not detected between replicate treatment systems. Mussels were killed more quickly in heat trials, with circulation also showing greater efficacy than the control (Figure 8, plot B). After 24h, all specimens were dead in heat and circulation treatments, and four mussels remained alive in the control.

BB treatments

Heat treatment increased the rate of *Mytilus* degradation relative to the control whilst circulation showed no effect over the first eight hours (Figure 8, plot C). A significant treatment effect was observed at 12 (ANOVA : $F_{(2,36)} = 55.84, P = <0.000$) and 24h (ANOVA : $F_{(2,42)} = 23.74, P = <0.000$). Heat trials removed significantly more mussel mass than control and circulation after 12 and 24h exposure. Circulation was not statistically different from the control at either time interval (Table 6). A significant difference was not detected between replicate treatment systems. *Mytilus* mortality occurred more quickly under heat treatment where 100% mortality was detected at 12h (Figure 8, plot C). After 24h all specimens were dead in circulation trials and two mussels remained alive in the control.

Discussion

The key advantage of descalers over other chemical solutions such as oxidising and non-oxidising biocides is their capacity to both chemically degrade and kill calcareous NIS. The degradation benefit has established descalers as an increasingly important remediation tool in certain high risk shipping operations where protocols demand complete removal of secondary and tertiary biofouling (Booth and Wells 2012) or 100% eradication of an NIS. This study aimed to test and compare a range of descalers to address the current paucity of knowledge regarding their efficacy and applications.

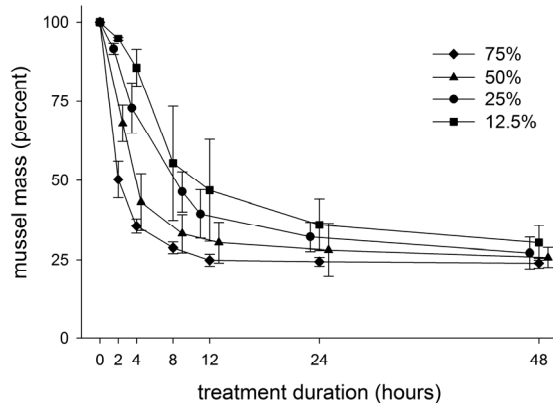


Figure 4. *Mytilus* degradation in 12.5, 25, 50 and 75% HCl-control treatments. Each point is the mean of five replicate mussels and plotted with ± 2 SE. Rate of decay after eight hours exposure: [75%] $m = -8.91$, [50%] $m = -8.35$, [25%] $m = -6.70$, [12.5%] $m = -5.57$.

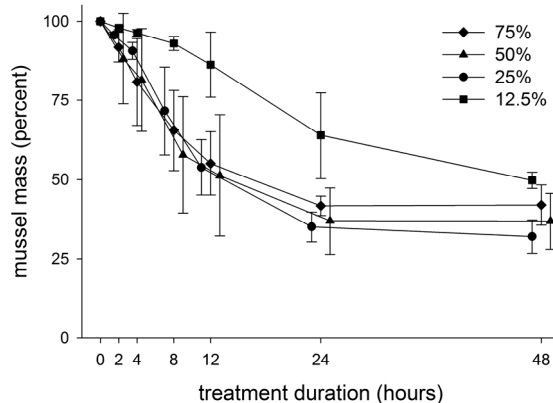


Figure 5. *Mytilus* degradation in 12.5, 25, 50 and 75% t7EP treatments. Each point is the mean of five replicate mussels and plotted with ± 2 SE. Rate of decay after eight hours exposure: [75%] $m = -8.91$, [50%] $m = -8.35$, [25%] $m = -6.70$, [12.5%] $m = -5.57$.

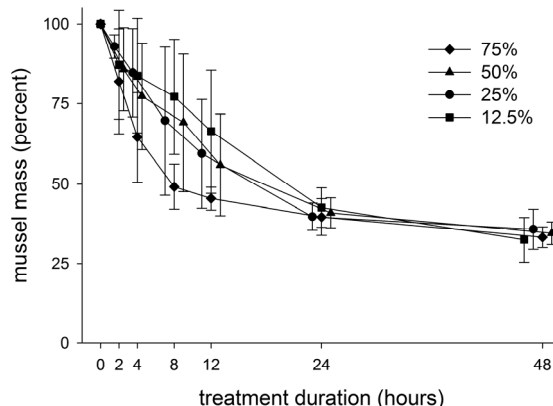


Figure 6. *Mytilus* degradation in 12.5, 25, 50 and 75% BB treatments. Each point is the mean of five replicate mussels and plotted with ± 2 SE. Rate of decay after eight hours exposure: [75%] $m = -6.36$, [50%] $m = -3.87$, [25%] $m = -3.79$, [12.5%] $m = -2.85$.

Table 5. Treatment concentrations which removed significantly more *Mytilus* mass for each descaler at 12, 24 and 48h. Only treatments at the lowest level of concentration (12.5%) were significantly less effective.

12h		24h		48h	
Sig. concentration comparison	P*	Sig. concentration comparison	P*	Sig. concentration comparison	P*
<i>RdM</i> [75%] > [12.5%]	0.021	<i>RdM</i> [25%] > [12.5%]	0.002	<i>RdM</i> [25%] > [12.5%]	0.006
<i>RdM</i> [50%] > [12.5%]	0.001	<i>t7EP</i> [50%] > [12.5%]	0.001	<i>t7EP</i> [25%] > [12.5%]	0.017
<i>RdM</i> [25%] > [12.5%]	0.024	<i>t7EP</i> [25%] > [12.5%]	0.001		
<i>DD</i> [50%] > [12.5%]	0.035				
<i>3HM</i> [50%] > [12.5%]	0.009				
HCl-control [75%] > [12.5%]	0.014				

Table 6. *3HM*, *t7EP* and *BB* multiple comparison summaries, showing the mean weight loss, SE and significance. Treatments not significantly different from each other are labelled with the same letter.

	<i>3HM</i>			<i>t7EP</i>			<i>BB</i>		
	Mean %	SE	Sig.	Mean %	SE	Sig.	Mean %	SE	Sig.
12h									
Control	51.12	2.39	A	12.34	1.91	A	42.81	3.50	A
Circulation	56.01	2.25	A	35.31	5.28	B	44.32	1.68	A
Heat	65.46	1.66	B	52.88	2.70	C	57.95	0.61	B
24h									
Control	65.19	1.68	A	32.16	3.23	A	50.58	2.29	A
Circulation	65.28	1.04	A	51.90	3.52	B	52.70	1.05	A
Heat	72.84	1.02	B	56.56	2.45	B	62.97	1.26	B

CaCO₃ degradation

The capacity for acid based descalers to react with CaCO_3 provides a fundamental measure of their efficacy. The observed association between descaler CaCO_3 degradation and starting pH for the HCl descalers, suggests that efficacy as a measure of CaCO_3 degradation may simply be a function of solution acidity for these products. The addition of excess CaCO_3 to descalers did not have a neutralising effect, with final pH recordings remaining acidic and highly variable between products (final pH 2.2-5.4). This indicates the presence of buffering agents and highlights the proprietary nature of descalers and their uncertain composition and chemistry. Measuring pH could be a useful strategy for gauging treatment progress (high reactivity being maintained by adding descaler when pH exceeds a predetermined threshold). However, the high variability observed between descaler pH values suggests it would be misleading to use pH to compare efficacy between descalers containing different acids (e.g., HCl, phosphoric, citric and lactic). The observed 29% difference between the lowest descaler degradation (*3H* at 111g/l) and highest (*DD* at 143g/l) has considerable implications for biosecurity treatments. Choice of product could

equate to large disparities in the volume (and potential cost) associated with successfully treating vessels with large internal seawater systems.

Knowledge of the capacity for descalers to degrade CaCO_3 could be applied directly in more simple operations (e.g., equipment soaking) if a target mass of calcareous biofouling can be reliably estimated. Whilst such calculations may be difficult to achieve in practice, they would enable treatments to be administered with confidence that sufficient descaler volume is present to be effective. In high risk situations, excess descaler could be applied to increase assurance that all NIS have been successfully eliminated. Conversely, having identified a target mass of CaCO_3 it should be possible to prevent overdosing, thereby reducing cost and risks to vessel piping integrity and/or the environment. Future investigations into the feasibility of accurately estimating CaCO_3 load within seawater systems is recommended, and could involve sub-sampling techniques supplemented by remote camera inspections.

Extrapolations of CaCO_3 degradation to the number of medium sized mussel shells helps place potential efficacy into context, however it is noted that complete shell dissolution is a highly conservative measure of efficacy as mortality precedes total loss of the shell.

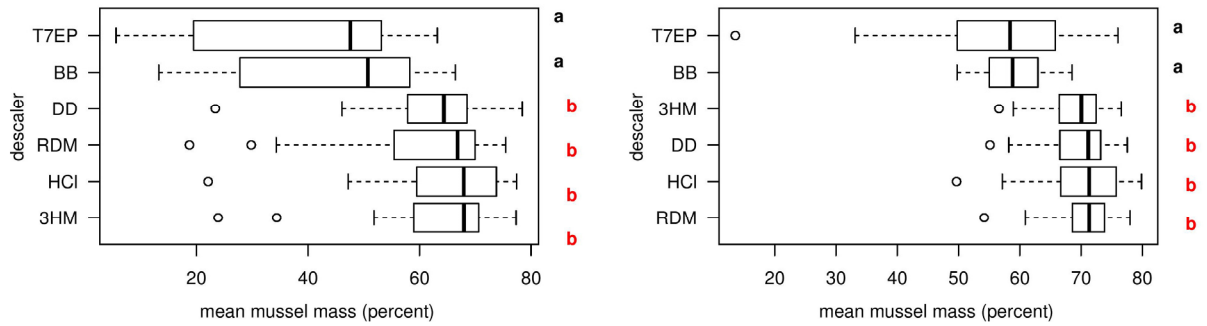


Figure 7. Multiple comparison boxplots showing a significant main effect of descaler type for *Mytilus* degradation at 12 (left) and 24h (right). Means not significantly different from each other are labelled with the same letter and colour.

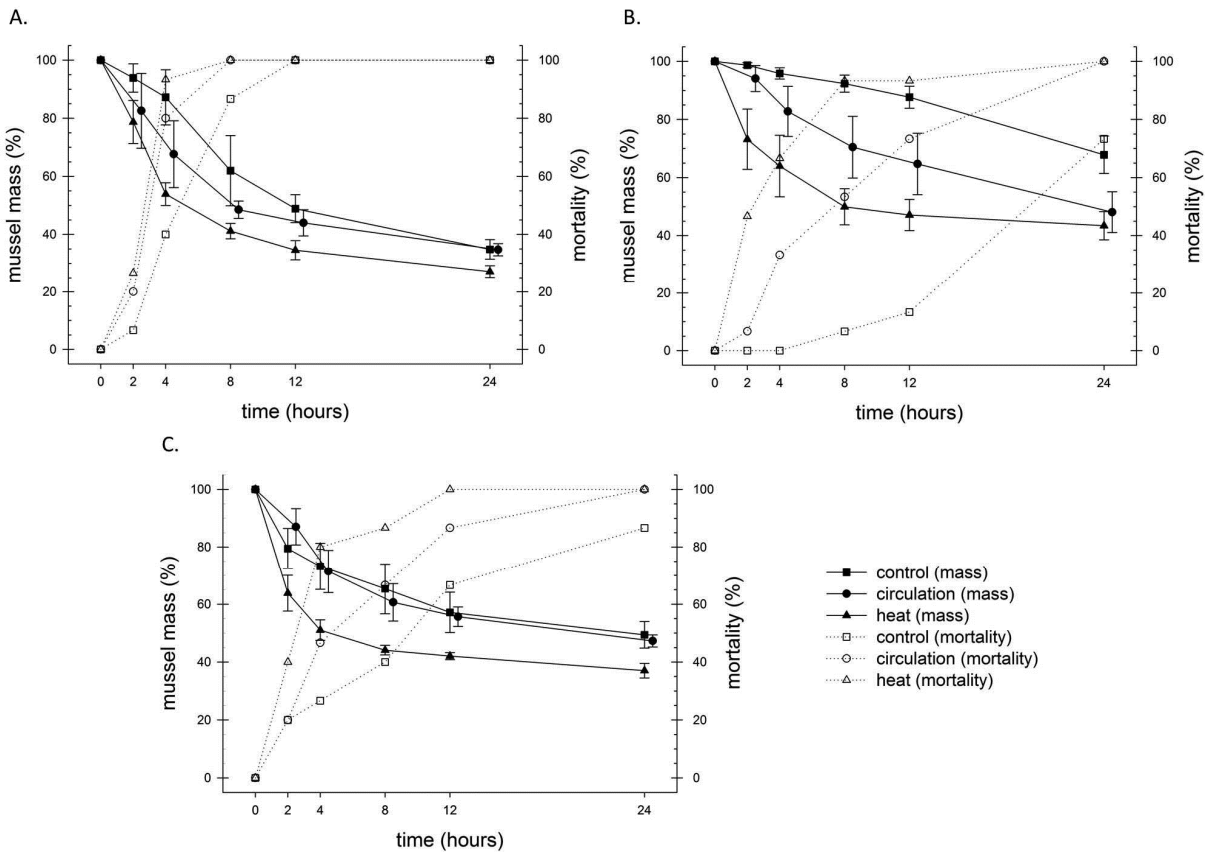


Figure 8. *Mytilus* weight loss and mortality in 3HM (A), t7EP (B) and BB (C), showing control (static, 11°C), circulation (0.25m/s, 11°C) and heat (static, 26°C) trials. Rate of decay after eight hours exposure: 3HM control $m = -4.76$, circulation $m = -6.43$, heat $m = -7.36$, t7EP control $m = -0.96$, circulation $m = -3.69$, heat $m = -6.26$, BB control $m = -4.34$, circulation $m = -4.92$, heat $m = -6.98$. Mortality is shown as the percentage of specimens dead over time, replicate trials (3) are pooled to give sample size of 15 mussels.

The effect of descaler type and concentration

Increasing descaler concentration had surprisingly little influence on mussel degradation. *Mytilus*

degradation in 25% treatments for all descalers was largely indistinguishable from 50 and 75% treatments. This result could have wide implications if scalable to the size of vessel treatments and applicable to other calcareous biofouling. A lack of evidence that

concentrations above 25% increase efficacy in this study may have been attributable to treatments exceeding a threshold in CaCO_3 – acid reactivity at a concentration between 12.5 and 25%.

Most *Mytilus* weight loss (> 90% of total degradation) occurred by 12h in $\geq 25\%$ HCl treatments and by 24h for *BB* and *t7EP*. During this time, high variability between replicates precluded unambiguous interpretation of the effect of concentration on the rate of mussel decay. High variability was caused by uneven *Mytilus* mortality driving disparities in the extent of shell fluid retention at the time of weighing. A clearer effect of concentration was observed post 12h when most organisms had died and variability had reduced. At this point, 25% treatments were just as effective as higher concentrations (50 and 75%) with the possible exception of *BB* treatments (where variability remained high).

Mytilus weight loss reached a plateau after 12h exposure to the HCl descalers suggesting that application of 25% *RdM*, *DD* or *3HM* for approximately 12h may be a good strategy for eliminating mussel biofouling at a level comparable to this study (e.g. single 50mm *Mytilus* per 0.4l). For the maritime industry, minimising treatment time is a high priority to ensure that a vessel can become fully operational as soon as possible. Considering that vessel treatments are commonly carried out for 24–48h at a concentration of 30–50% (Coutts and Gust pers. obs.), it will be important to substantiate the potential efficacy of 12 hour 25% HCl descaler treatments at the vessel scale.

Mytilus degradation was slower in 12.5% treatments but by 48h no significant differences in mussel weight were detected between 12.5 and 75% for all descalers. This suggests that increasing concentration much beyond the minimum required for complete CaCO_3 degradation is likely to have little effect on total degradation potential given sufficient treatment time. This conclusion is supported by *Mytilus* periostracum, viscera and byssus threads remaining intact after 48h of descaler exposure even in 75% treatments. After 48h all concentration levels achieved very similar mean mussel weight reductions, the exceptions being 25% *RdM* and *t7EP* treatments which removed a greater mass than 12.5%. This result is difficult to interpret and may be an artefact of small sample sizes. In practical instances where longer treatment times are available (i.e., 48h) and the type and extent of calcareous biofouling is known, dosing a seawater system with a 12.5% descaler could achieve equivalent results to 75% treatments. Clearly this would offer considerable benefits to industry via reduced costs and decreased risk of damage to vessel equipment.

Unfortunately, in some parts of the world protocols for the correct disposal of descaler waste into onshore management facilities are absent or ignored, and effluent is released directly into the marine environment. In these instances, achieving comparable efficacy with lower descaler concentrations would reduce potential impact to coastal ecosystems and port/harbour water quality. Furthermore, the high potency of descalers observed in this study suggests a potential for recycling of treatment solution, particularly in biosecurity operations that target only small numbers of NIS.

The HCl-control treatment was just as effective as the HCl descalers, suggesting that the presence of descaler constituents additional to HCl do not improve mussel degradation under the experimental conditions. However, greater evidence of a concentration effect in HCl-control treatments could suggest that in descaler treatments, additional constituents (e.g., catalysts) increased the rate of reactivity at lower concentrations, facilitating the maintenance of a similar, rapid reactivity for 25–75% treatments. Such a hypothesis could be investigated in future research.

The products *BB* and especially *t7EP* were less effective at degrading *Mytilus* than the HCl descalers. Slower CaCO_3 reactivity in these two products is likely attributable to lower hydrogen ion concentrations on account of phosphoric (*BB*), citric and lactic acids (*t7EP*) being weak acids. However, evidence of reaction products calcium citrate and/or calcium lactate remaining associated with test specimens subject to *t7EP*, suggest that *Mytilus* degradation was underestimated and/or accumulated reaction products could have reduced acid- CaCO_3 reactivity for this product. Higher variability in mussel weight observed in *BB* and *t7EP* treatments, (especially from 0–24h) reflects slower mortality rates prolonging the duration of fluid retention in some specimens. Particular circumstances could warrant application of a less aggressive descaler. If vessel piping and components are old and corroded, applying a treatment with a lower concentration of hydrogen ions may help reduce reaction with metal oxides, preventing structural damage and even potential leakage which has been observed in some industry applications (Gust pers. obs.).

Descalers have received limited attention as a tool for marine biosecurity in the literature. *Rydlyme*[®] was screened as a tool for treating oysters in small vessels (Neil and Stafford 2005) and mussels in seawater systems (Lewis and Dimas 2007). Neil and Stafford (2005) concluded that *Rydlyme*[®] was ineffective for killing the oyster *Saccostrea glomerata* (Gould, 1850) but did not report on CaCO_3 dissolution. Lewis and Dimas (2007) observed that

25% *Rydlyme*[®] and 5% HCl were needed to remove the shell of one mussel (a weight reduction reported as approximately 50%) leaving only organic matter. In comparison, this study showed that 25, 50 and 75% *RdM* (estimated 11% lower CaCO₃ degradation potential than *Rydlyme*), and the HCl-control removed approx. 70% of the initial weight of *Mytilus* after 24h. Lewis and Dimas (2007) regarded effectiveness as complete dissolution of the calcareous valves and concluded that treating seawater systems with *Rydlyme*[®] was impractical because the volume of descaler needed to eliminate a vessel mussel infestation would be too high. Data presented in phase two (and contemporary industry practice) is inconsistent with this conclusion. The treatment volume used in phase two (0.4l) can be cautiously extrapolated to the scale of vessel seawater systems to predict a theoretical capacity of the number of similar sized (approx. 50mm) mussels that might show comparable degradation. If an internal seawater system capacity of 10,000l is applied, it can be theorized that 25,000 mussels might show comparable degradation to any phase two treatment (i.e., approximately 70% weight reduction if treated with 25% *RdM* for eight hours). Such an extrapolation of the experimental conditions is clearly not applicable in practice where factors such as non-target mineral deposition and biofouling, difficulties in maintain a homogenous treatment solution and factors affecting CaCO₃ degradation (e.g., temperature, pressure and salinity) will impact treatment efficacy. Nevertheless, when appropriate concentrations and treatment durations are applied, the capacity for all descalers tested to compromise infestations of mussels inhabiting seawater systems and niche areas, appears considerable.

The effect of temperature and circulation

The application of heat to a descaling treatment was predicted to increase the rate of *Mytilus* degradation in response to a higher energy of reactants and subsequent increase in the frequency of molecular collisions. This effect was observed in phase three, where warming treatments from 11 to 26°C significantly increased *Mytilus* weight reduction for each of the three descalers tested over 24h exposure. Observations of remnant calcareous shell and intact viscera, periostracum and byssus indicate that this effect was caused by an increase in the rate of CaCO₃-acid reactivity rather than tissue hydrolysis.

In warmer treatments an acceleration in *Mytilus* mortality relative to the control was also observed and is likely attributable to an increase in the rate of shell dissolution, facilitating quicker exposure of

Mytilus tissue to descaler chemicals. Such an increase in efficacy was particularly apparent in *BB* and *t7EP* treatments where the rate of mortality increased markedly from the control. Improving efficacy by warming appropriate descalers could be advantageous if limited time is available for treatment or a particularly high risk NIS is identified. For the maritime industry, minimising the time a vessel is waylaid can reduce costs considerably. Descaling treatments carried out at high latitudes are clearly candidates for heating. Authorities should also consider that treatment schedules proven to be successful in tropical regions or summer conditions will be less effective in temperate waters or in winter, in which case a judicious approach might involve heating or increasing treatment time.

The feasibility of maintaining large volumes of descaler at elevated temperatures within internal seawater systems will vary considerably depending on the vessel design and area requiring treatment. Introduction of portable immersion heaters into a seawater system could be feasible in specific circumstances or descaler could be heated prior to introduction into a target area. Whilst warming a treatment may increase efficacy and reduce treatment time, corrosion of piping, damage to vulnerable components and increased health and safety risks may also rise. It is therefore vital that the manufacturer's Material Safety Data Sheets (MSDS) are consulted prior to application. Most descaler manufacturers claim that their products are highly effective, safe to use on most vessel materials, non-toxic to human health, safe for the environment and highly biodegradable. Such claims need to be investigated as it seems unlikely they are all simultaneously achievable.

Exposure to circulating *t7EP* increased the rate of *Mytilus* weight loss. This may have been in response to the maintenance of a homogenous solution which prevented the localised depletion of acid or accumulation of reaction products. Some evidence of increased weight loss was apparent for *Mytilus* in circulating *3HM*, but not *BB*. The small size of experimental systems (approximately eight litres) may have enabled easy diffusion of *BB* in control trials preventing circulation from showing an effect. A clear effect of circulation observed in *t7EP* trials (and suggested in *3HM*), supports a hypothesis that treatment mixing may be particularly important in large vessel seawater systems where stratification and slow diffusion are likely to reduce the efficacy of static treatments.

The most effective descaling treatments identified in phase three were *3HM* heat and circulation trials; after eight hours, weight loss was observed at

approximately 59 and 51% respectively, and mortality was 100%. Whilst no studies in the available literature have assessed mussel degradation through the chemical reaction with strong acids, mortality results from this study indicate comparable or higher efficacy than a range of oxidising and non-oxidising treatments from the literature (Table 1). An exception is the especially rapid mortality observed by Piola and Hopkins (2012); these authors successfully killed comparable sized *Mytilus* to those examined in the present study by heating seawater to 42.5°C for 10 minutes. Comparatively, this study focused on *Mytilus* degradation through time, thus mortality results are not discrete and provide a conservative approximation of mortality.

Evidence of *Mytilus* periostracum and shell separating from the viscera during heat and circulation trials (also observed in *t7EP* trials in phase two) indicate that at a fine scale, some tissue breakdown may have been evident. However, similar to phase two, *Mytilus* tissue remained intact and surprisingly undamaged. Tissue resistance to hydrolysis in descaler acid may be an important consideration for management when planning a vessel treatment. The collection of organic debris at filtration points may be necessary for heavily biofouled internal systems and would require sufficient circulation velocity. Removal of large amounts of organics would improve system efficiency and allow examination of material for viable NIS.

Conclusions and recommendations

Increases in maritime trade and offshore resource extraction will ensure that a high rate of NIS incursions continue into the foreseeable future (Meyerson and Mooney 2007; Thomaz et al. 2015). Ongoing demand exists for effective marine biosecurity tools to help manage biofouling and eliminate NIS from internal seawater systems and vessel niche areas. Experimental evidence here indicates that current industry application of $\geq 50\%$ descaler concentrations is too high under most conditions, and comparable efficacy could be attained with $\leq 25\%$ treatments. For operations requiring mussels to be eliminated as quickly as possible, HCl descalers appear likely to achieve greater efficacy than phosphoric and acid-surfactant treatments. Concerns regarding toxicity and corrosiveness to vessel materials remain pertinent and require future investigation across multiple descaler types. Increasing the scale of trials to entire vessel treatments will be important to assess the efficacy of reduced concentrations arising from laboratory trials in this study.

The application of descalers for the purpose of “completely” removing biofouling to meet legislative requirements is misleading, as results here clearly showed that descalers do not completely dissolve mussel organic material, even at 75% concentrations. In contrast, achieving 100% mortality and calcium carbonate removal with descalers is entirely feasible. Current industry practice regularly involves circulating descaler treatments in vessel piping; this is recommended and could be applied to soak treatments of biofouled equipment and niche areas with the use of portable pumps. Warming treatments maybe feasible in certain circumstances; nevertheless concerns remain regarding the compatibility of gaskets and other vessel materials, particularly if the structural integrity of isolated piping and materials cannot easily be verified. The treatment of internal seawater systems with large volumes of proprietary acids to remove and kill biofouling should be a last-resort strategy. Continued research into more effective biofouling prevention and environmentally safe and cheap biofouling remediation is needed.

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