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Stay clean: direct steam exposure to manage biofouling risks

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Abstract

Biofouling by marine organisms can result in a variety of negative environmental and economic consequences, with decontamination procedures remaining problematic, costly and labour-intensive. Here, we examined the efficacy of direct steam exposure to induce mortality of selected biofouling species: *Mytilus edulis*; *Magallana gigas*; *Semibalanus balanoides*; *Fucus vesiculosus*; and an *Ulva* sp. Total mortality occurred at 60-sec of steam exposure for *M. edulis* and juvenile *M. gigas*, at 30-sec for *S. balanoides*, while 300-sec was required for adult *M. gigas*. Application of steam reduced the biomass of *F. vesiculosus* and significantly reduced *Ulva* sp. biomass, with complete degradation being observed for *Ulva* sp. following 120-sec of exposure. Accordingly, it appears that steam exposure can cause mortality of biofouling organisms through thermal shock. Although preliminary, our novel and promising results suggest that steam applications could potentially be used to decontaminate niche areas and equipment.

Keywords:

Biosecurity, decontamination, invasive species, spread-prevention, macroalgae, bivalve, barnacle

1. Introduction

The natural colonisation of submerged surfaces by adhering organisms, i.e. biofouling, can result in substantial economic and environmental impacts for a variety of marine-based industries, such as shipping, desalination/purification and aquaculture (Schultz 2007; Nurioglu et al. 2015). In particular, biofouling presents a range of design and operational challenges for marine renewable energy devices, and is considered a priority research topic for this emerging industry (Loxton et al. 2017). Moreover, as artificial coastal infrastructure such as docks and floating pontoons can provide suitable habitats for biofouling species, commercial harbours and recreational marinas may act as source habitats for general nuisance species as well as damaging invaders, thus facilitating their further spread (Ferrario et al. 2017; Marić et al. 2017; Iacarella et al. 2019). Biofouling organisms and biofilm layers may also harbour and facilitate the further spread of damaging pathogenic microorganisms (Kent 1992; Douglas-Helders et al. 2003; Drake et al. 2005).

Biofouling by invasive alien species (IAS) can adversely impact marine biodiversity, ecosystem functioning, human health and marine-based industries, e.g. fishing and tourism (Bax et al. 2003; Molnar et al. 2008; Lovell & Drake 2009). Once established at a new location, the complete eradication of aquatic invaders is often unfeasible, while on-going population suppression is costly (Piria et al. 2017; Coughlan et al. 2018b). The adherence of IAS to equipment, watercraft and moveable infrastructure, especially within niche areas that are difficult to inspect or decontaminate such as sea chests, chain lockers, pipework, intake grates, and fishing nets (Sylvester & MacIsaac 2010; Moser et al. 2017; de Castro et al. 2018), represents an important pathway for the translocation of damaging marine invaders, which continue to spread at an unprecedented rate worldwide (Ricciardi et al. 2016; Seebens et al. 2016; Iacarella et al. 2019).

To date, mitigation of biofouling has largely been managed through the use of preventative biocidal (toxic) paints, chemical cleaning agents and physical decontamination methods, such as encapsulation, brushing, scraping, high-pressure washing, and hand removal (Hopkins et al. 2016; Growcott et al. 2017; Silva et al. 2019). More recently, following the high-profile international ban on the use of tributyltin compounds in biocides, there has been increased focus on the use of environmentally-friendly non-toxic preventative coatings (Silva et al. 2019). However, as discussed by Hopkins et al. (2016), despite meaningful advances in prevention of biofouling, not all surfaces are amenable to antifouling paints, e.g. fishing equipment, aquaculture infrastructure, mooring lines, and drilling rigs. Accordingly, numerous other decontamination methods have been proposed as suitable alternatives, including exposure to desiccation (Hopkins et al. 2016), low salinity (de Castro et al. 2018), ultraviolet light (Titus & Ryskiewich 1994), acid or alkaline soaking treatments (Rolheiser et al. 2012) and heated water (Piola & Hopkins 2012). However, the efficacy of these treatments is often unknown (Hopkins et al. 2016; Growcott et al. 2017). Many removal techniques also rely on visual

inspection to confirm the absence of biofouling organisms following decontamination treatments, however, visual inspection of some sections of equipment may not be possible, and decontamination methods alone (e.g. desiccation, brushing, scraping, high-pressure washing, hand-removal) will not necessarily result in complete propagule removal or mortality of adhering organisms (Rothlisberger et al. 2010; Coughlan et al. 2018a). Accordingly, innovative, cost-effective and environmentally-friendly, yet highly efficacious techniques, are urgently required (Growcott et al. 2017; Crane et al. 2019; Coughlan et al. 2019).

Notably, the thermal application of steam treatments has been suggested as one such method, and may be a particularly beneficial addition to out of water dock-side decontamination procedures, especially for niche areas such as internal surfaces of pipework (Jones & Little 1990; Growcott et al. 2017; Cahill et al. 2019). However, the physiological tolerances of biofouling organisms, i.e. their susceptibility to thermal shock, to the innovative and novel approach of steam decontamination remains poorly studied.

In this study, we thus examined the efficacy of brief out of water direct steam exposure to induce mortality of selected common biofouling species, including two bivalve molluscs, *Mytilus edulis* and invasive *Magallana gigas* (formerly *Crassostrea gigas*), one barnacle species, *Semibalanus balanoides*, and two alga species, *Fucus vesiculosus* and an *Ulva* sp. We hypothesised that direct steam exposure will induce substantial, if not complete, mortality of the targeted biofouling species, dependent on exposure time.

2. Methods

2.1 Specimen collection and maintenance

All organisms were collected in Northern Ireland (NI) from a variety of locations (Table 1), and transported in source water to the Queen's Marine Laboratory (QML), Portaferry, NI. Specimens were maintained in outdoor flow-through seawater tanks at ~12 °C, for a period of 48–72 hrs prior to commencement of the experiments.

2.2. Experimental steam exposure

Each species was independently exposed to steam treatments. Experimental groups included: ten adult *M. edulis* (total shell length: 35.27 – 44.95 mm; shell length per group, mean $\pm$ SE: 40 $\pm$ 0.2 mm); ten juvenile *M. gigas* (14.9 – 15 mm); a 5 cm² area with $\geq$ 80 % cover of *S. balanoides* (71.5 $\pm$ 2.6 individuals); subsamples of *F. vesiculosus* (60.5 $\pm$ 0.3 g); and subsamples of *Ulva* sp. (35.3 $\pm$ 0.3 g). Due to their relatively large size, adult specimens of *M. gigas* (90.94 – 146.61 mm: 110.8 $\pm$ 3.3 mm) were each steamed individually. Experimental groups were placed on a flat plastic board and

were directly exposed to a continuous jet of steam (≥ 100 °C; 350 kPa; Karcher® SC3 Steam Cleaner) at a distance of 2-3 cm from the spout of the device for: 10, 30, 60, 120 or 300-sec (Table 1). All treatments were replicated in triplicate for each species examined, except adult *M. gigas*, which were replicated six times. The jet of steam was manoeuvred along the entire upwards facing area of each experimental group, i.e. specimen or group of specimens, for the duration of the assigned exposure time by repeatedly moving the jet of steam in an oscillating manner. Control groups were allowed to air dry for the maximum 300-sec treatment period. Following steam exposure, specimens were allowed to cool for a 15-min period and were then placed in outdoor flow-through seawater tanks at ~ 12 °C. A 24 hr recovery period was allowed for *M. edulis*, *M. gigas* and *S. balanoides*, after which mortality was assessed. Specimens were considered dead if they were gaping, or if they offered no resistance to being teased apart with tweezers and did not reclose. Changes in mass following a 12 or 5 day recovery period were recorded for *F. vesiculosus* and the *Ulva* sp., respectively. Percentage tissue degradation of the *Ulva* sp. was also visually recorded in relation to the surface area.

2.3 Data analyses

Data analyses were performed using R v3.4.4 (R Core Development Team 2018). Differences in mean bivalve shell lengths according to steam treatment groups were analysed using one-way Kruskal-Wallis rank sum tests. Mortality of *M. edulis*, *M. gigas* and *S. balanoides* was examined with respect to steam exposure time using generalised linear models assuming binomial error distributions. Bias-reduced penalised-likelihood logistic regression was implemented where perfect separation occurred (Firth 1993). Likelihood ratio tests between nested models were used to infer significance levels according to steam treatment. Differences in *F. vesiculosus* and *Ulva* sp. weights (natural-log transformed) before and after steam treatments were examined using analysis of variance, given residuals were normally distributed (Shapiro-Wilk test, $P > 0.05$) and homoscedastic (Bartlett's test, $P > 0.05$). *Ulva* sp. tissue degradation was additionally analysed with respect to steam treatment using beta regression (Cribari-Neto and Zeileis 2010), following appropriate transformations (Smithson and Verkuilen 2006). Where necessary, Tukey *post-hoc* tests were used to deduce pairwise comparisons among steam treatment levels.

3. Results

3.1 *Mytilus edulis*

Shell lengths of bivalves did not differ significantly according to steam treatment groups ($\chi^2 = 3.99$, $df = 5$, $P > 0.05$). Steam exposure significantly increased mortality of mussels *Mytilus edulis* ($\chi^2 = 192.58$, $df = 5$, $P < 0.001$; Fig. 1A). Survival of mussels was 100 % in control groups, and total

mortality was evidenced at steam exposures of 60-sec and longer. *Post-hoc* comparisons between treatment groups are shown on Fig. 1A.

3.2 Juvenile *Magallana gigas*

For juvenile *M. gigas*, exposure to steam also caused a significant increase in mortality ($\chi^2 = 135.83$, $df = 5$, $P < 0.001$; Fig. 1B). Survival of juvenile *M. gigas* in control groups was 100 % and total mortality was observed following steam exposures of 60-sec and longer. *Post-hoc* comparisons between treatment groups are shown on Fig. 1B.

3.3 Adult *Magallana gigas*

Shell lengths of bivalves did not differ significantly according to steam treatment groups ($\chi^2 = 7.61$, $df = 3$, $P > 0.05$). Although adult *M. gigas* mortality was significantly increased following exposure to steam ($\chi^2 = 24.94$, $df = 3$, $P < 0.001$), with total mortality observed after 300-sec exposures, no significant pairwise difference occurred between treatment groups ($P > 0.05$; Fig. 1C).

3.4 *Semibalanus balanoides*

Steam treatment significantly increased mortality of barnacles ($\chi^2 = 856.73$, $df = 4$, $P < 0.001$; Fig. 1D). Whilst control survival exceeded 90 % in all replicates, total mortality was observed in barnacles treated with steam for 30-sec or longer. *Post-hoc* comparisons between treatment groups are shown on Fig. 1D.

3.5 *Fucus vesiculosus*

Changes to weights of the examined brown fucoid alga were not significantly influenced by treatment with steam ($F_{4, 10} = 3.26$, $P > 0.05$; Fig. 2A). However, whilst weight differences among control, 10, 30 and 60-sec treatment groups were similar, weights following 120-sec steam exposures were reduced substantially and consistently.

3.6 *Ulva* sp.

Overall, weights of the filamentous green alga were significantly reduced after steam exposure ($F_{4, 10} = 9.25$, $P < 0.01$; Fig. 2B). *Post-hoc* comparisons between treatment groups are shown on Fig. 2B.

Steam treatment also significantly increased visually-scaled degradation of the *Ulva* sp. ($\chi^2 = 45.41$, $df = 4$, $P < 0.001$; Fig. 2C). Here, all steam exposure times degraded the filamentous green alga significantly more than controls (all $P < 0.001$), and up to complete degradation was observed following 120-sec treatments. *Post-hoc* comparisons between treatment groups are shown on Fig. 2C.

213 4. Discussion

214 The direct application of steam caused complete mortality for all examined biofouling
 215 invertebrates within exposure times of 30–60-sec, except adult *M. gigas*, which required 300-sec of
 216 steam exposure for total mortality to be achieved. For the macroalgae, following steam exposure both
 217 species displayed a reduction in relative biomass, with *Ulva* sp. being completely degraded following
 218 120-sec of steam exposure. Accordingly, when taken together, these results indicate that the rapid
 219 decontamination of equipment and niche areas could be achieved with brief out of water steam
 220 exposure. Although it appears that longer exposure times will be needed to kill larger adult organisms,
 221 reasonably frequent applications will likely prevent the development of adult life stages and reduce
 222 the detrimental impacts of biofouling species. Interestingly, although an overall trend of decreased
 223 relative biomass was observed for *F. vesiculosus*, some specimens exposed to short treatment times of
 224 ≤ 60 -sec also displayed increased biomass, an interesting response that warrants further investigation.
 225 Equally, *F. vesiculosus* presented the largest surface area of all examined organisms (PWSJ and NEC
 226 *per. obs.*), and therefore would not have experienced the same intensity of steam per unit area of its
 227 external surfaces as the other examined organisms. Yet our results for macroalgae are indicative only,
 228 and a more thorough assessment is required. For example, warm-water acclimated organism may
 229 experience a reduced impact of thermal shock. For an improved assessment, longer steam exposure
 230 times, warm-water acclimated organisms, and an extended recovery period should be used to
 231 thoroughly assess the effect of steam exposure on *F. vesiculosus*. In addition, the examination in-water
 232 steam delivery systems and their efficacy is required. Overall, however, the application of rapid steam
 233 treatments could potentially be used to prevent the establishment and spread of nuisance organisms
 234 and damaging invaders.

235 The application of heat treatments in the form of heated water has previously been successfully
 236 used to exterminate damaging biofouling organisms, prevent the establishment of complex fouling
 237 assemblages, and decontaminate niche areas (Inglis et al. 2012; Piola & Hopkins 2012; Cahill et al.
 238 2019). Yet, the application of steam could possibly be more efficacious than hot water treatments in
 239 certain scenarios, as additional thermal energy in the form of latent heat will be released with the
 240 condensation of steam into water (see 1st Law Thermodynamics). In essence, whilst remaining at a
 241 constant temperature, this physical state change from a gas to a liquid will transfer a greater amount of
 242 thermal energy to target organisms. Also, heated water can be impractical or cost-prohibitive when
 243 applied at a large-scale, particularly when the water temperature needs to be consistently maintained
 244 within a set range to achieve uniform mortality of target species (Inglis et al. 2012; Piola & Hopkins
 245 2012; Growcott et al. 2017). But the economic cost of steam treatments will need to be clarified. In
 246 addition, the post-treatment release of large volumes of hot water can have ecological consequences,

including thermal shock-induced mortality of non-target organisms, and the dispersion of biofouling organisms that have survived treatment exposure. Although in-water steam treatments will have similar operational concerns, such incidents of thermal pollution and species dispersion are potentially more avoidable with the use of steam during dry dock-side treatment, which will mostly dissipate into the surrounding air and is not likely to flush living organisms through pipework unless highly pressurised. Equally, although the use of broad-spectrum aquatic disinfectants has been proposed as a rapid method for achieving enhanced biosecurity (Rolheiser 2012; Cuthbert et al. 2018; 2019), the release of large volumes of chemical solutions following treatment could have similarly prohibitive environmental consequences.

Although steam treatments used here will likely kill adhering organisms through intense thermal shock, steam will not necessarily remove the residual biomass, unless delivered under pressure. That being said, groups of steam-treated *S. balanoides* appeared to detach from stone surfaces much more readily than non-treated specimens when handled for assessment by experimental operators (PWSJ and NEC *pers. obs.*). Despite this, dead organisms will eventually decompose, while the direct application of pressurised jets (e.g. through a lance like device) of steam may prove effective for both in-water and dry dock-side targeted removal of biofouling organisms, particularly when synergistically combined with additional cleaning methods, such as hand removal, brushing or scraping. Ensuring total mortality of adhering invasive species prior to removal attempts, could be especially beneficial for reducing opportunities for further invader spread. Installation of steam cleaning devices at points of entry (e.g. marinas, harbours and boat ramps) may facilitate utilisation of this simple and environmentally-friendly, but highly efficacious biosecurity protocol (Crane et al. 2019). These steam decontamination facilities could mimic the design of car-wash stations (Coughlan et al. 2019), and could potentially be designed for self-service or operated by a trained attendant (Crane et al. 2019).

Importantly, however, once the required intensity of steam to efficaciously kill target organisms has been deciphered, visual inspection of surfaces will no longer be necessarily required. For example, within niche areas such as the internal surface of pipework, and complex structures like sea chests, temperature sensors could be used to ensure an adequate duration of steam exposure to cause complete mortality of any biofouling organisms. Essentially, steam generators could be used to inject steam into pipework and other niche areas (Growcott et al. 2017), to facilitate the improved decontamination of large and more structurally complex equipment at high pressures and temperatures, e.g., 10–12 Bar; ≥ 180 °C (Crane et al. 2019). Under such conditions the efficaciousness of steam treatments would likely be further enhanced, as the target organisms would be exposed to increased levels of thermal energy, retained as latent heat within the pressurised and hotter steam treatments. However, the development of operational treatments for internal niches will require further assessment, especially in relation to heat distribution, and risk of damage to vessel components and existing anti-foul coatings. In particular, further research to assess the effects of steam applications on the integrity of antifouling

coatings, fibreglass and epoxy resin surfaces of hulls, niche areas and equipment is also required. Although our novel series of experiments represent a realistic and promising starting-point, an improved understanding of the physiological tolerances of biofouling organisms to steam exposure is required. In particular, our experiments require up-scaling to higher densities of organisms, complex fouling assemblages, differential life stages, the assessment of impacts to non-target organisms and water quality parameters, and testing under realistic *in situ* scenarios.

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6. Author contributions

PWSJ and NEC proposed the study; PWSJ and NEC designed the experiment; PWSJ, NEC and GYWV conducted the experiment; RNC and NEC performed data analysis; all authors contributed to writing the manuscript, which was led by NEC.

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Figure 1: Mean percentage mortality ($\pm$ SE) of *Mytilus edulis* (A: groups of 10; $n = 3$), juvenile and adult *Magallana gigas* (B & C: groups of 10 or 1; $n = 3$ or 6, respectively) and *Semibalanus balanoides* (D: 5 cm² area with ≥ 80 % cover; $n = 3$) following exposure to direct steam treatments for up to 300-sec. All species were assessed following a 24-hr recovery period. Shared letters indicate no significant difference ($P > 0.05$).

Figure 2: Mean percentage relative biomass change ($\pm$ SE) for *Fucus vesiculosus* (A: 60.5 ± 0.3 g; $n = 3$) and a collection of *Ulva* sp. (B: 35.3 ± 0.3 g; $n = 3$) following exposure to direct steam treatments for up to 120-sec. Mean percentage degradation ($\pm$ SE) for *Ulva* sp. is also shown (C). Each species was assessed following a 12 or 5 day recovery period, respectively. Shared letters indicate no significant difference ($P > 0.05$).

467 Figure 1:

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